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MATERNAL AND CHILD HEALTH, NUTRITION, AND HIV

EMILY ROSE SMITH

A Dissertation Submitted to the Faculty of

The Harvard T.H. Chan School of Public Health

in Partial Fulfillment of the Requirements

for the Degree of *Doctor of Science*

in the Department of Global Health and Population

Harvard University

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Dissertation Advisor: Dr. Wafaie Fawzi **Emily Rose Smith**

Maternal and Child Health, HIV, and Nutrition

Abstract

Reducing maternal and child mortality was established as a global priority with the signing of the Millennium Declaration in September 2000. Neonatal vitamin A supplementation and very early breastfeeding initiation are scalable interventions which may improve infant survival. Although breastfeeding has proven benefits for infant health, the potential health consequences of breastfeeding for HIV-infected women are not well studied.

In paper one, **"The effect of neonatal vitamin A supplementation on morbidity and mortality at 12 months: A randomized trial"**, we assessed the efficacy of neonatal vitamin A supplementation (NVAS) in reducing infant morbidity and mortality. Using data from an individually randomized clinical trial of 31,999 infants in Tanzania, we found that NVAS did not affect the risk of death or the incidence of morbidities. However, we noted that postpartum maternal vitamin A supplementation modified the effect of neonatal vitamin A supplementation on infant mortality.

In paper two, **"Effect of delayed breastfeeding initiation on infant survival: a systematic review and meta-analysis"**, our objective was to synthesize the evidence regarding the association between breastfeeding initiation time and infant morbidity and mortality. We pooled five studies, including 136,047 infants. We found a clear dose-response relationship; the risk of neonatal mortality increased with increased delay in breastfeeding initiation. We found a similar pattern when the analysis was restricted to exclusively breastfed infants or low birthweight infants. There was limited evidence regarding the association between breastfeeding initiation time and infant morbidity and growth. We concluded that health policy frameworks

and models to estimate newborn and infant survival should consider the independent survival benefit associated with early initiation of breastfeeding.

In paper three, **"Breastfeeding and Maternal Health among HIV-infected Women in Tanzania",** our objective was to assess the relationship between infant feeding practices and the incidence of maternal mortality, morbidity, and indicators of poor nutritional status from six weeks to two years postpartum in a prospective cohort of Tanzanian women living with HIV. We concluded that breastfeeding may be associated with mixed health outcomes. Additional research should investigate whether HIV-infected women require nutritional support, in addition to antiretroviral therapy, during and after lactation.

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Title: The effect of neonatal vitamin A supplementation on morbidity and mortality at 12 months: A randomized trial

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Running title: Neonatal Vitamin A Supplementation in Tanzania

Abbreviations: Appropriate for gestational age (AGA), small for gestational age (SGA), food frequency questionnaire (FFQ), human immunodeficiency virus (HIV), vitamin A deficiency (VAD), neonatal vitamin A supplementation (NVAS), maternal vitamin A supplementation (MVAS), acute respiratory infection (ARI), Institute of Medicine (IOM), recommended daily allowance (RDA), vitamin A (VA)

Trial Registry: Australian New Zealand Clinical Trials Registry (ANZCTR) - ACTRN12610000636055

Abstract

Background: Neonatal vitamin A supplementation (NVAS) is an intervention hypothesized to reduce infant morbidity and mortality. The objective of this study was to assess the efficacy of neonatal vitamin A supplementation in reducing infant morbidity and mortality and assess potential sources of heterogeneity of the effect of NVAS.

Methods: We completed an individually randomized, double-blind, placebo-controlled trial in Tanzania. Infants were randomized within three days of birth to a single dose of vitamin A (50,000 IU) or placebo. We assessed infants at one and three days after supplementation, as well as one, three, six, and 12 months after supplementation. We included all live births in the analysis and used relative risks and 95% confidence intervals to assess the risk of mortality and hospitalization by 12 months. We used general estimating equations to assess the incidence of morbidities during infancy.

Results: A total of 31,999 infants were enrolled in the study between August 2010 and March 2013. At 12 months, vitamin A did not reduce all-cause infant mortality (RR 1.04; 95% CI 0.92-1.16), nor affect hospitalization (RR 1.09; 95% CI 0.97-1.22) or all-cause morbidity (RR 1.00; 95% CI 0.96-1.05). Postpartum maternal vitamin A supplementation modified the effect of neonatal vitamin A supplementation on mortality at 12 months (p-value, test for interaction = 0.04). Among infants born to women who received a mega-dose of vitamin A after delivery, NVAS appeared to increase the risk of death (RR 1.12; 95% CI 0.98-1.29), while the risk of death among infants born to women who did not receive a mega-dose was reduced (RR 0.86;

95% CI 0.70-1.06). We noted no effect modification of the effect of NVAS by infant gender, birthweight, or maternal HIV status.

Conclusion: NVAS did not affect the risk of death or incidence of common childhood morbidities. However, this study sheds light on potential sources of heterogeneity of the effect of neonatal vitamin A supplementation which should be further examined in a pooled analysis of all NVAS trials.

Keywords: Neonatal Vitamin A Supplementation, Infant Mortality, Hospitalization, Morbidity, Tanzania

Key Messages

- The recent completion and analysis of the three largest neonatal vitamin A supplementation trials (NEOVITA) found conflicting results regarding the efficacy of this intervention in reducing infant morbidity and mortality. A meta-analysis combining this data with that of seven previous trials found important heterogeneity in mortality effects.
- Overall, we found no evidence that neonatal vitamin A supplementation in Tanzania reduces the risk of mortality, hospitalization, or morbidity during infancy.
- Data from this trial suggests that maternal vitamin A status may modify the effect of neonatal vitamin A supplementation.

Introduction

Despite improvements in child survival in the past four decades, an estimated 6.3 million children under the age of five died in 2013 (1). Although child mortality continues to decline, there has been slower progress in reducing infant mortality. Interventions to reduce neonatal and infant mortality are needed, and there is evidence that vitamin A supplementation can reduce morbidity and mortality among children aged 6 to 59 months (2). A number of trials to assess the efficacy of neonatal vitamin A supplementation have had conflicting findings. While some trials have shown clear benefit associated with supplementation (3, 4), others suggest a null or possibly harmful effect (5, 6). The reason for qualitative differences in the effect of neonatal vitamin A supplementation between these trials is not well understood.

The efficacy of neonatal vitamin A supplementation may be modified by baseline maternal or infant characteristics. A meta-analysis including all published trials to date (7), suggested that geographic region, which is highly correlated with the population prevalence of vitamin A deficiency, may explain the heterogeneity of trial results. Specifically, trials performed in Asia (also classified as having moderate or severe maternal vitamin A deficiency) (3, 4, 8-10) had a 13% reduced risk of mortality from supplementation to six months (RR: 0.87, 95% CI: 0.78, 0.96), whereas African trials (countries also classified as having mild or no maternal deficiency) (5, 6, 11-13) showed a 10% increased risk of mortality associated with neonatal vitamin A supplementation (RR: 1.10, 95% CI: 1.00,1.21). A trial in Guinea Bissau among normal birthweight infants (given 50,000 IU or 25,000 IU vitamin A) and a trial in Zimbabwe among HIV-infected women also found no effect of neonatal vitamin A supplementation on mortality (14, 15). The differences in findings between trials conducted in Asia and Africa may also be

attributable to variability in the prevalence of HIV, given that vitamin A supplementation has been associated with higher risk of mother-to-child transmission of HIV (16). Low birthweight or prematurity are also potential effect modifiers, as vitamin A deficiency is more prevalent among these infants, and these infants may benefit from supplementation (17). Child sex and timing of vaccination have also been hypothesized to modify the effect of vitamin A supplementation on mortality (18).

We examined the effects of neonatal vitamin A supplementation on the incidence of all-cause and cause-specific infant morbidity as measured by hospitalization and caregiver report of illness in the NEOVITA Tanzania trial (5). Additionally, we assessed whether the efficacy of neonatal vitamin A supplementation in reducing infant morbidity and mortality was modified by baseline maternal and infant characteristics.

Methods

Study Design and Population

The data was collected as part of an individually randomized, placebo controlled trial conducted in Tanzania from August 2010 through March 2014. Infants were randomized at home or health facility to receive a mega-dose of vitamin A (50,000 IU) or placebo on the day of birth or within three days (n=31,999). Details regarding the randomization, blinding, intervention, and follow up are described elsewhere (5, 19). Infants were eligible for randomization if they were able to feed orally, the family intended to stay in the study area for at least 6 months, and parents provided informed consent. Follow up data were collected during home visits one and three days after supplementation, as well as one, three, six, and 12 months after supplementation. The study

protocol was approved by the Institutional Review Boards of the Harvard School of Public Health, Ifakara Health Institute, Medical Research Coordinating Council of Tanzania, and by the WHO Ethical Review Committee. The trial is registered at Australian New Zealand Clinical Trials Registry (ANZCTR) -ACTRN12610000636055.

Outcome Definitions

The primary outcomes of interest included mortality, hospitalization, and morbidity between supplementation and one year (360 days). We assessed cause-specific hospitalization and symptom-specific morbidity occurring between supplementation and one year (360 days). We also present morbidity and mortality outcomes assessed six months (180 days) as secondary endpoints.

Potential Effect Modifiers

Potential effect modifiers of the effect of vitamin A on mortality specified *a priori* in the trial protocol include infant sex (male or female), birthweight (<2500 g and ≥2500 g), received maternal large-dose vitamin A supplementation (yes or no), and socioeconomic status. Two additional maternal characteristics were considered post-hoc as potential effect modifiers including: maternal vitamin A intake classified by the Institute of Medicine (IOM) recommended daily allowance (RDA) [below RDA (<700 μg/day), within RDA (700 to <3,000 μg/day), above RDA (≥3,000 μg/day)] (20) and maternal HIV status at delivery (positive or negative). Additionally, we combined information from the maternal large-dose vitamin A supplementation data (yes or no) and the maternal vitamin A intake data (below RDA or within RDA) to create "maternal vitamin A status" categories: 1) High (maternal supplementation + within RDA), 2) Medium-High

(maternal supplementation + below RDA), 3) Medium-Low (no maternal supplementation + within RDA), 4) Low (no maternal supplementation + below RDA).

Data Collection and Categorization

Infant death and date of death were ascertained by research staff at home visits. Hospitalization was defined as an inpatient admission to the hospital since the last home visit, which was transcribed from the child health card or as reported by the mother in the case of missing child health card. Research staff recorded the admission date and reason for hospitalization as either a) acute lower respiratory infection or pneumonia, b) diarrhea, c) fever or malaria, or d) other reason. Data from the first case of hospitalization was used in the analysis. Caregiver report of morbidity was assessed by one month caregiver recall at one, three, six, and 12 month home visits. During home visits, research staff asked caregivers if the child had any of the following symptoms in the past month: cough; refusal to eat, drink, or breastfeed; fever; difficulty in breathing; chest retraction; convulsions; vomiting; diarrhea.

Child sex and birthweight were assessed by a study nurse or dosing supervisor at randomization. Postpartum maternal vitamin A supplementation (yes or no) was assessed by research staff at randomization and again at 1 month postpartum. Maternal education and pregnancy history were assessed by field interviewers during a baseline interview. A wealth index was generated based on household ownership of assets, and households were categorized into wealth quintiles based on this index. For all women enrolled in the trial during the first year of recruitment, maternal dietary intake was assessed around the time of birth by trained field interviewing using a semi-quantitative food frequency questionnaire (FFQ) (21-25). Based on monthly recall of 108

commonly consumed foods, we calculated average daily servings and translated this data into nutrient intake based on the Tanzania Food Composition Tables (26). Maternal HIV status (positive, negative, unknown) was abstracted from the delivery ward record books for the subset of women who delivered at health facilities which maintained registry books where HIV status at delivery was recorded. The time of breastfeeding initiation and whether colostrum was given to the infant was assessed by a study nurse or dosing supervisor at the time of dosing and again at one and three days after dosing if the infant had not initiated breastfeeding at the time of dosing. The age of the child at dosing was calculated as the number of hours between the time of birth and time of dosing. Gestational age at birth was calculated based on maternal report of last menstrual period. Size for gestational age at birth was calculated based on INTERGROWTH standard (27). Gestational age and size for gestational age was categorized in the following four groups: preterm (<37 weeks gestation) small for gestations age (<10^{th} percentile in weight for gestational age), preterm appropriate for gestational age (≥10^{th} percentile in weight for gestational age), term (≥37 weeks) small for gestational age, or term appropriate for gestational age.

Statistical Analysis

The cohort was characterized using baseline data regarding household, maternal, and infant characteristics using means or proportions for continuous and categorical data respectively. Including all live-births, we estimated risk ratios and their corresponding 95% confidence intervals to assess the relationship between vitamin A supplementation and the risk of mortality and hospitalization within 12 months. We used log binomial general estimating equations (GEEs) with an exchangeable working covariance matrix to assess the relationship between vitamin A

supplementation and morbidity within 12 months, allowing for repeated episodes of morbidity for each child. For the subgroup analyses, potential effect modification by maternal and infant characteristics (including infant sex, birthweight, maternal vitamin A supplementation, maternal HIV status, and maternal vitamin A intake) was assessed using the likelihood ratio test comparing the full and reduced log binomial regression models. We also present mortality, hospitalization, and morbidity analyses within the first 6 months (180 days) as secondary analyses. P-values less than 0.05, in conjunction with 95% confidence intervals and the magnitude of effect estimates, were used to identify results of importance. Analysis was completed using SAS version 9.2 (Cary, NC).

Results

Among 32,843 infants assessed for eligibility, a total of 31,999 infants were enrolled in the study between August 2010 and March 2013. Among those enrolled, 15,995 were randomized to the vitamin A group and 16,004 were randomized to the placebo group (Figure 1.1). Between supplementation and one year (360 days), 779 (4.9%) infants were lost to follow up in the vitamin A group and 762 (4.8%) infants were lost to follow up in the placebo group.

The mean age at supplementation was 15.5 (SD 12) hours in the vitamin A group and 15.4 (SD 12) in the placebo group; about 80% of infants were supplemented within 24 hours after birth. Mean birthweight was 3.02 (SD 1) kilograms in both groups. More than 75% of all women received a mega-dose of vitamin A after delivery (as part of standard of care in Tanzania), and the median value of dietary intake of vitamin A for mothers was 940 µg/day (IQR 574-1389).

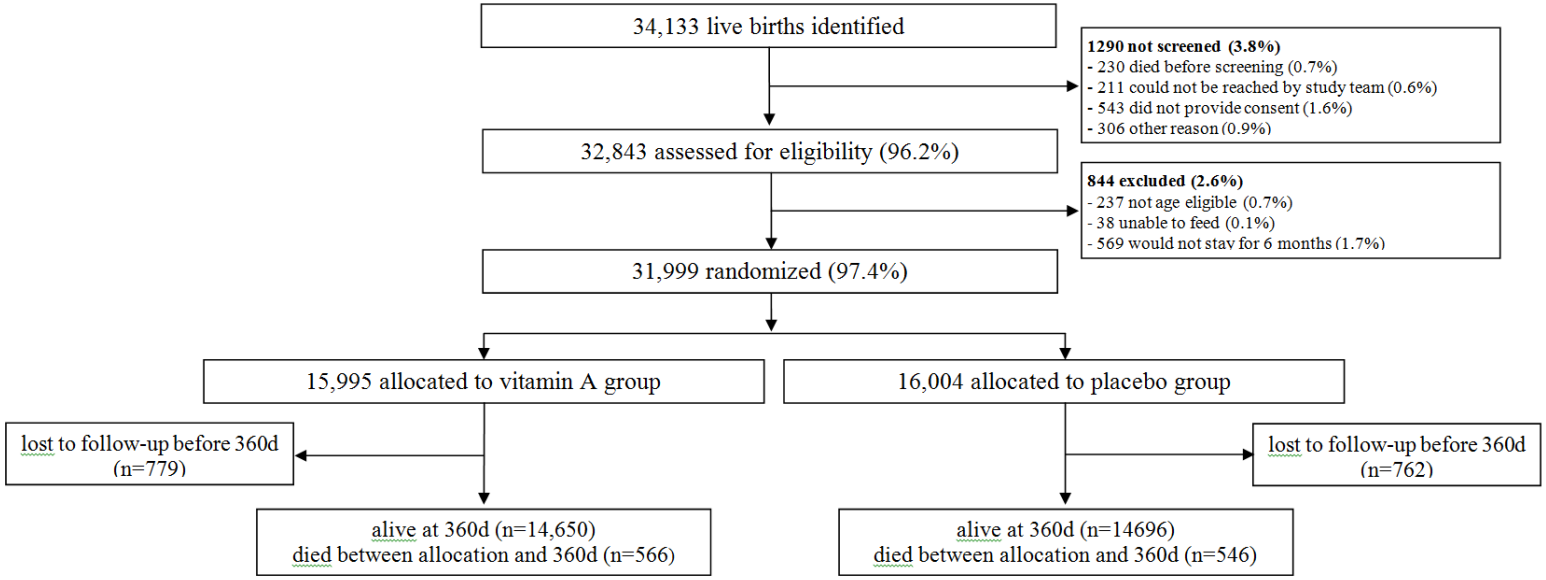

Figure 1.1 Trial Profile

Table 1.1 Baseline demographic and health characteristics of mothers and infants (n=31999)

	Vitamin A Group (n=15995) n (%)	Placebo Group (n=16004) n (%)
Male sex	8443 (52.8)	8340 (52.1)
Birthweight <2500 grams	1908 (11.9)	1974 (12.3)
Maternal education[1]		
None	1335 (9.0)	1310 (8.8)
Primary	11988 (80.9)	12066 (81.3)
Secondary	1491 (10.1)	1472 (9.9)
Received maternal vitamin A supplementation	12122 (76.8)	12091 (76.6)
Colostrum given to infant	15390 (97.4)	15416 (97.6)
Parity[1]		
1	3872 (29.5)	3843 (29.2)
2-3	5785 (44.1)	5745 (43.7)
≥4	3455 (26.3)	3551 (27.0)
Time of dosing[1]		
<24 hours	12774 (79.9)	12824 (80.1)
24-47 hours	2939 (18.4)	2881 (18.0)
≥48 hours	282 (1.8)	299 (1.9)
Gestational age & size for gestational age[1]		
Term AGA	6768 (66.4)	6738 (65.8)
Term SGA	1705 (16.7)	1688 (16.5)
Preterm AGA	1670 (16.4)	1771 (17.3)
Preterm SGA	46 (0.5)	46 (0.4)
Initiation of breastfeeding[1]		
≤1 hour	14083 (88.3)	14169 (88.9)
2-23 hours	1726 (10.8)	1670 (10.5)
≥24 hours	138 (0.9)	105 (0.7)
Maternal HIV status		
HIV infected	139 (0.9)	155 (1.0)
HIV uninfected	2747 (17.2)	2708 (16.9)
Not assessed	13109 (82.0)	13141 (82.1)
Maternal vitamin A dietary intake		
<700 µg/day	1018 (6.4)	1007 (6.3)
700 to <3000 µg/day	1950 (12.2)	1969 (12.3)
≥3000 µg/day	28 (0.2)	32 (0.2)
Not assessed	12999 (81.3)	12996 (81.2)

[1]These numbers do not add to 100% due to missing data

Treatment groups were well balanced in regards to demographic, maternal, and infant characteristics (Table 1.1).

At 12 months, vitamin A did not reduce all-cause infant mortality. There were 566 (3.5%) deaths in the vitamin A group and 546 (3.4%) deaths in the placebo group (RR 1.04; 95% CI 0.92-1.16). NVAS did not reduce all-cause hospitalizations or morbidity. There were 596 (3.7%) hospitalizations in the vitamin A group and 547 (3.4%) hospitalizations in the placebo group (RR 1.09; 95% CI 0.97-1.22). NVAS did not affect the incidence of common childhood illnesses through 12 months, with a similar number of caregiver reported morbidity events in both groups (RR 1.00; 95% CI 0.96-1.05) (Table 1.2). Similar results were observed in secondary analyses of mortality, hospitalization, and morbidity within six months of age (Supplementary Table 1A).

All potential effect modifiers were assessed, and mortality at 12 months did not differ by any characteristic (including sex, birthweight, maternal HIV status, maternal vitamin A dietary intake, and socioeconomic status (data not shown)), except by postpartum maternal vitamin A supplementation (Table 1.3). The relative risk of mortality from supplementation to 12 months comparing NVAS to placebo for those whose mothers received a large dose of vitamin A was 1.12 (95% CI 0.98-1.29), while the relative risk for those infants whose mother did not receive postpartum vitamin A supplementation was 0.86 (95% CI 0.70-1.06) (p-value, test for interaction = 0.04). A similar trend was observed by overall maternal vitamin A intake (post-partum vitamin A supplementation and dietary intake). Among children born to mothers with high overall vitamin A dietary intake (who received a post-partum vitamin A supplement and had vitamin A dietary intake within the recommended daily allowance (RDA) according to their food

Table 1.2. The effect of neonatal vitamin A supplementation on infant mortality, hospitalization, and morbidity at 12 months.

	Vitamin A		Placebo		Risk Ratio[1] (95% CI)	p-value
	n[2]	N[2]	n[2]	N[2]		
Mortality (All-Cause)	566	15995	546	16004	1.04 (0.92,1.16)	0.54
Hospitalization (All-Cause)	596	15995	547	16004	1.09 (0.97,1.22)	0.14
ALRI	215	15995	197	16004	1.09 (0.90,1.32)	0.37
Diarrhea	142	15995	127	16004	1.12 (0.88,1.42)	0.36
Fever	360	15995	325	16004	1.11 (0.96,1.29)	0.17
Other	136	15995	122	16004	1.12 (0.87,1.42)	0.38
Morbidity (All-Cause)	4129	38880	4125	38962	1.00 (0.96,1.05)	0.88
Diarrhea	1006	38880	1016	38962	0.99 (0.91,1.09)	0.86
Diarrhea & Vomitting	578	38880	557	38962	1.04 (0.92,1.18)	0.53
Diarrhea & Vomitting & Fever	518	38880	502	38962	1.03 (0.91,1.18)	0.61
Diarrhea & Refused Feeding	449	38880	441	38962	1.02 (0.89,1.18)	0.77
Fever	2543	38880	2521	38962	1.01 (0.96,1.07)	0.69
Fever & Cough	1008	38880	1036	38962	0.98 (0.89,1.07)	0.59
Fever & Difficulty Breathing	583	38880	552	38962	1.06 (0.93,1.20)	0.38
Fever & Cough & Difficulty Breathing	505	38880	469	38962	1.08 (0.94,1.23)	0.28
Cough	2125	38880	2159	38962	0.99 (0.93,1.05)	0.68

[1]Risk ratio for morbidity outcomes were estimated by GEE log binomial model with an exchangeable working covariance matrix.

[2] n is the number of events (*i.e.* deaths, hospitalizations, morbidity cases). N is the number of infants for mortality and hospitalization analysis. N is the number of household visits for morbidity analysis.

Table 1.3. The effect of neonatal vitamin A supplementation on infant mortality (0-12 months), stratified by subgroup. (n=31999)

	Number of newborns supplemented				Risk Ratio (95% CI)	p-value	p-value test for interaction
	Vitamin A		Placebo				
	n[1]	N[1]	n[1]	N[1]			
Overall [N=31,999]	566	15995	546	16004	1.04 (0.92,1.16)	0.54	
Sex [N=31,994]							
Male	312	8443	324	8340	0.95 (0.82,1.11)	0.52	0.09
Female	254	7549	222	7662	1.16 (0.97,1.39)	0.10	
Birthweight [N=31,983]							
<2500 grams	138	1908	120	1974	1.19 (0.94,1.51)	0.15	0.21
≥2500 grams	428	14077	426	14024	1.00 (0.88,1.14)	0.99	
Maternal Vitamin A Supplementation [N=31,559]							
Yes	408	12122	362	12091	1.12 (0.98,1.29)	0.10	0.04
No	152	3662	178	3684	0.86 (0.70,1.06)	0.16	
Maternal HIV Status [N=5,749]							
HIV infected	15	139	10	155	1.67 (0.78,3.60)	0.18	0.31
HIV uninfected	65	2747	59	2708	1.09 (0.77,1.54)	0.64	
Maternal Vitamin A Dietary Intake [N=6,004]			1				
<700 µg/day	36	1018	41	1007	0.87 (0.56,1.35)	0.53	
700 to <3000 µg/day	75	1950	65	1969	1.17 (0.84,1.61)	0.36	0.15
≥3000 µg/day	0	28	2	32	-		

[1] n is the number of deaths. N is the number of infants

Table 1.4. The effect of neonatal vitamin A supplementation on infant mortality (0 to 12 months), stratified by maternal vitamin A supplementation and maternal vitamin A dietary intake. (n=5820)

		Number of newborns				Risk Ratio (95% CI)	p-value test for trend	p-value test for interaction
		Vitamin A		Placebo				
		n[1]	N[1]	n[1]	N[1]			
Overall		109	2911	106	2909	1.02 (0.79,1.34)	0.84	
Maternal vitamin A supplementation & dietary intake [2]								
High	Maternal supplementation + adequate VA dietary intake	52	1418	39	1415	1.33 (0.88-2.00)	0.04	0.07
	Maternal supplementation + inadequate VA dietary intake	27	750	27	720	0.96 (0.57-1.62)		
	No maternal supplementation + adequate VA dietary intake	22	487	26	506	0.88 (0.51-1.53)		
Low	No maternal supplementation + inadequate VA dietary intak	8	256	14	268	0.60 (0.26-1.40)		

[1] n is the number of deaths. N is the number of infants

[2] Vitamin A (VA). Adequate VA dietary intake is defined as <700 µg/day. Inadequate VA dietary intake is defined as 700 to <3000 µg/day.

frequency questionnaire), NVAS was associated with an increased risk of infant mortality (RR 1.33; 95% CI 0.88-2.00). Children born to women with the lowest maternal vitamin A status (no maternal supplementation and vitamin A intake below the RDA according to their food frequency questionnaire) had a 40% reduced risk of death associated with NVAS (RR 0.60; 95% CI: 0.26-1.40) (Table 1.4). Including maternal vitamin A status as an ordinal covariate in the model resulted in a p-value for the test for trend of 0.04 and a p-value for the test of interaction of 0.07. Qualitatively similar results were observed for effect modification by maternal supplementation and overall vitamin A dietary intake in secondary analyses restricted to events in the first six months of life (Supplementary Table 1B, Supplementary Table 1C).

Discussion

We found that neonatal vitamin A supplementation did not reduce infant mortality or the incidence of hospitalization. Our findings are in accord with those from a systematic review (conducted prior to the conclusion of the three recent NVAS trials) that found no evidence of an effect of NVAS on hospitalization (RR: 0.75; 95% CI 0.26 to 2.16), although the authors noted that the quality of available evidence was low (28). However, NVAS was associated with an increased risk of hospitalization at six months in the companion NEOVITA trial in Ghana (RR 1.11; 95% CI 1.00 to 1.22) (6). Similarly, a neonatal vitamin A supplementation trial in south India, as well as a trial among older children in Indonesia, found an increased incidence of acute respiratory infection among children supplemented with vitamin A (VA) (29, 30). Additionally, research among children hospitalized for ARI found that vitamin A supplementation increased the severity of symptoms (31-33). Increased severity of illness is hypothesized to be the result of the proinflammatory immune responses associated with VA (34). We did not find any evidence

of effect modification of the effect of neonatal vitamin A supplementation on mortality at six and 12 months by child sex. This is consistent with a meta-analysis completed prior to completion of the three recent NEOVITA trials (35).

We found there was no difference in the incidence of common childhood morbidities at six or 12 months when comparing infants randomized to receive vitamin A at birth and those who were not. Our results regarding hospitalization and morbidity are contrary to the hypothesis that vitamin A supplementation affects mortality by reducing the incidence or severity of infant morbidity (30, 36, 37). This hypothesis was biologically plausible, as vitamin A is known to play an important role in immune function (34). Human and animal models illustrate that vitamin A helps maintain and improve epitheilia integrity (including those in the respiratory and gastrointestinal tract) and may down-regulate production of proinflammatory cytokines in response to specific pathogens. Furthermore, observational data has shown maternal vitamin A deficiency (VAD) is associated with increased morbidity in children. For example, maternal night blindness during pregnancy was associated with an increased risk of diarrhea, dysentery, and acute respiratory infections (ARI) between birth and six months of age among a cohort of infants in South India (38). Other observational research confirms that VAD children are more likely to be carriers of *Streptococcus pneumoniae* (*Spn*) (39-41) (an important cause of ARI) and have increased rates of ARI morbidity and mortality (42, 43). However, randomized trials in Bangladesh and south India found no difference between the vitamin A and placebo groups in the proportion of infants carrying *Spn* at three and four months respectively (44, 45). Our results are consistent with a meta-analysis including neonatal vitamin A supplementation and maternal vitamin A supplementation (MVAS) plus NVAS trials, which found no effect on morbidity

between supplementation and six months (46). Similar morbidity results have been documented among trials assessing the efficacy of vitamin A supplementation among children age six to 56 months (29, 36, 47, 48).

We found evidence that maternal vitamin A status may modify the effect of neonatal vitamin A supplementation. In this trial NVAS appeared to be beneficial for infants born to mothers who were not supplemented with vitamin A, while infants born to women who received postpartum vitamin A supplementation demonstrated a higher risk of mortality associated with neonatal vitamin A supplementation. This finding is consistent with the hypothesis that the prevalence of vitamin A deficiency is an important source of heterogeneity among the recent NVAS trials (7). The NEOVITA trials in Ghana and Tanzania both suggested the possibility of an increased risk of mortality associated with NVAS, and the prevalence of vitamin A deficiency (<0.70 μmol/L serum retinol A) among a subset of mothers enrolled in the trials was low in both settings (Ghana: < 3% VAD (6) ; Tanzania: 8% VAD (5)). Additionally, the prevalence of postpartum vitamin A supplementation was high in both settings (Ghana: 50% supplemented; Tanzania: 76% supplemented). In contrast, about 12% of women in the companion trial in India trial were vitamin A deficient and no mothers received postpartum vitamin A supplementation, and in this context NVAS was associated with a reduced risk of mortality from supplementation to six months (3). A factorial designed trial of maternal and neonatal vitamin A supplementation trial was conducted in Bangladesh and found no interaction between maternal and neonatal vitamin A supplementation, although the statistical power to detect a moderate or small degree of interaction was limited (8). Similarly, a factorial designed trial in Zimbabwe found no interaction between maternal and neonatal vitamin A supplementation among an analysis of HIV-uninfected

mothers (13). Notably, as of 2011 the World Health Organization no longer recommends maternal postpartum vitamin A supplementation (49).

We did not find clear evidence that maternal HIV status modified the effect of neonatal vitamin A supplementation. However, there was a trend showing increased risk of mortality associated with NVAS among infants born to HIV-infected mothers. HIV-exposed neonates supplemented with vitamin A were two times more likely than those in the placebo group to die before six months, and they had a 67% increased risk of death by one year. Although our cohort for this subgroup analysis was small, our trial is one of two NVAS trials in which the HIV status of mothers was known. In a trial conducted in Zimbabwe among HIV-infected women that randomized woman and infant pairs to a) postpartum maternal vitamin A supplementation (MVAS) alone, b) NVAS alone, c) both MVAS and NVAS, or d) placebo, the unadjusted hazard ratio for child mortality from enrollment to 24 months, comparing neonatal vitamin A supplementation (without maternal vitamin A supplementation) to placebo was 1.21 (95% CI: 0.99-1.46, p-value = 0.05) (15). Using fixed-effects meta-analysis, the pooled effect size comparing NVAS to placebo, among infants born to HIV-infected women in the Tanzania and Zimbabwe trials, is 1.23 (95% CI: 1.02-1.49) (Supplementary Figure 1A). This suggests that NVAS may be harmful for children born to HIV-infected mothers. In the Zimbabwe trial, there was an increased risk of mother to child transmission of HIV among the NVAS group, compared to placebo (15). Similarly, a randomized trial conducted among HIV-infected mothers in Tanzania found vitamin A was associated with a higher risk of mother to child transmission of HIV (16). As a result, increased mother to child transmission may partially explain why we found a trend of increased risk of death associated with neonatal vitamin A supplementation among infants born to HIV-infected mothers.

There are several strengths of this study. First, this is the largest randomized trial of neonatal vitamin A supplementation in sub-Saharan Africa. Detailed baseline data were collected for all mothers and infants, comprehensive longitudinal data about infant illness were collected, and loss to follow-up was less than 5% at one year. Furthermore, hospitalizations were assessed from the child medical card throughout the course of infancy, thus providing a high quality measure of severe morbidity. Finally, these data provide perhaps the only opportunity to look at maternal HIV status as a modifier of the effect of neonatal vitamin A supplementation, as additional trials of NVAS among children born to HIV-infected mothers are unlikely. One limitation of this research is that we used caregiver recall to assess morbidity. Further, mothers were asked to report morbidity within the last 30 days at one, three, six, and 12 months and as a result, there are no caregiver reports of morbidity for more than half of infancy. Thus, the data reported here are likely an underestimate of the true incidence of infant morbidities. Nevertheless, the analytical methods we used are well suited for data collected by an unbalanced visit schedule. Finally, although this is a large randomized trial, we may lack statistical power to detect moderate to small degree of interaction in all subgroups. Further, maternal vitamin A dietary intake and HIV status data were collected in only a subset of women enrolled in the trial. Our finding of effect modification by maternal vitamin A status may have occurred by chance, and it is important these results are replicated in other trials.

Overall, our findings are consistent with the hypothesis that NVAS does not affect the risk of infant mortality or the incidence of common childhood morbidities. Furthermore, this study generates additional hypotheses about the potential sources of heterogeneity of the effect of

neonatal vitamin A supplementation on infant health, which should be further examined in a pooled analysis of all NVAS trials.

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Author Contributions

1. designed research (project conception, development of overall research plan, and study oversight); **ES, AM, HM, WF**
2. conducted research (hands-on conduct of the experiments and data collection); **ES, AM, SM, CS, RN, HM, WF**
3. analyzed data or performed statistical analysis; **ES, CS, DS**
4. wrote paper and have reviewed final paper (only authors who made a major contribution); **all authors**
5. had primary responsibility for final content; **ES, WF**

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Supplementary Table 1A. The effect of neonatal vitamin A supplementation on six-month mortality, hospitalization, and morbidity (0-6 months).

	Vitamin A		Placebo		Risk Ratio[1] (95% CI)	p-value
	n[2]	N[2]	n[2]	N[2]		
Mortality (All-Cause)	407	15995	372	16004	1.09 (0.95,1.26)	0.20
Hospitalization (All-Cause)	332	15995	303	16004	1.10 (0.94,1.28)	0.24
ALRI	129	15995	113	16004	1.14 (0.89,1.47)	0.30
Diarrhea	62	15995	43	16004	1.44 (0.98,2.13)	0.06
Fever	193	15995	170	16004	1.14 (0.93,1.39)	0.22
Other	78	15995	76	16004	1.03 (0.75,1.41)	0.87
Morbidity (All-Cause)	2070	24947	2097	25014	0.99 (0.93,1.06)	0.81
Diarrhea	388	24947	441	25014	0.88 (0.77,1.02)	0.09
Diarrhea & Vomitting	219	24947	261	25014	0.84 (0.70,1.02)	0.08
Diarrhea & Vomitting & Fever	204	24947	244	25014	0.84 (0.69,1.03)	0.09
Diarrhea & Refused Feeding	196	24947	222	25014	0.89 (0.72,1.09)	0.26
Fever	1143	24947	1169	25014	0.98 (0.91,1.07)	0.71
Fever & Cough	445	24947	466	25014	0.96 (0.84,1.10)	0.59
Fever & Difficulty Breathing	278	24947	292	25014	0.96 (0.80,1.14)	0.61
Fever & Cough & Difficulty Breathing	228	24947	239	25014	0.96 (0.79,1.17)	0.68
Cough	1118	24947	1143	25014	0.98 (0.91,1.07)	0.72

[1]Risk ratio for morbidity outcomes were estimated by GEE log binomial model with an exchangeable working covariance matrix.

[2] n is the number of events (*i.e.* deaths, hospitalizations, morbidity cases). N is the number of infants for mortality and hospitalization analysis. N is the number of household visits for morbidity analysis.

Supplementary Table 1B. The effect of neonatal vitamin A supplementation on six-month mortality (0-6 months), stratified by subgroup. (n=31999)

	Number of newborns supplemented				Risk Ratio (95% CI)	p-value	p-value test for interaction
	Vitamin A		Placebo				
	n[1]	N[1]	n[1]	N[1]			
Overall [N=31,999]	407	15995	372	16004	1.09 (0.95,1.26)	0.20	
Sex [N=31,994]							
Male	233	8443	215	8340	1.07 (0.89,1.29)	0.47	0.73
Female	174	7549	157	7662	1.12 (0.91,1.39)	0.28	
Birthweight [N=31,983]							
<2500 grams	106	1908	87	1974	1.26 (0.96,1.66)	0.10	0.27
≥2500 grams	301	14077	285	14024	1.05 (0.90,1.24)	0.53	
Maternal Vitamin A Supplementation [N=31,559]							
Yes	284	12122	249	12091	1.14 (0.96,1.35)	0.13	0.36
No	117	3662	119	3684	0.99 (0.77,1.27)	0.93	
Maternal HIV Status [N=5,749]							
HIV infected	9	139	5	155	2.01 (0.69,5.85)	0.19	0.21
HIV uninfected	45	2747	45	2708	0.99 (0.65,1.49)	0.95	
Maternal Vitamin A Dietary Intake [N=6,004]							
<700 µg/day	22	1018	30	1007	0.73 (0.42,1.25)	0.24	
700 to <3000 µg/day	57	1950	48	1969	1.20 (0.82,1.75)	0.35	0.17
≥3000 µg/day	0	28	1	32	-		

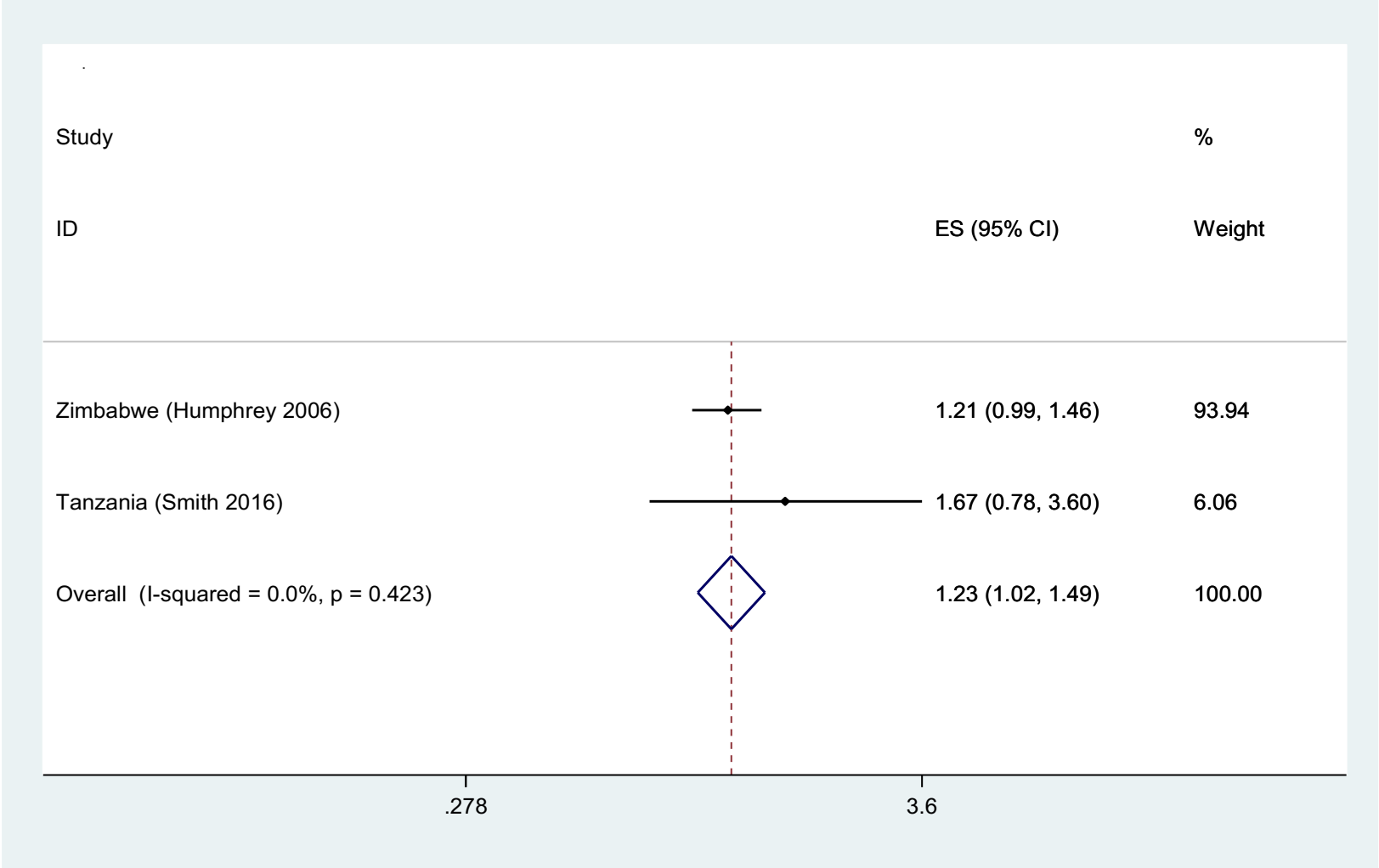
[1] n is the number of deaths. N is the number of infants

Supplementary Table 1C. The effect of neonatal vitamin A supplementation on six-month mortality (0 to 6 months), stratified by maternal vitamin A supplementation and maternal vitamin A dietary intake. (n=5820)

		Number of newborns				Risk Ratio (95% CI)	p-value test for trend	p-value test for interaction
		Vitamin A		Placebo				
		n[1]	N[1]	n[1]	N[1]			
Overall		77	2911	78	2909	0.99 (0.72-1.35)	0.93	
Maternal vitamin A supplementation & dietary intake [2]								
High	Maternal supplementation + adequate VA dietary intake	37	1418	26	1415	1.42 (0.86-2.33)	0.004	0.07
	Maternal supplementation + inadequate VA dietary intake	15	750	18	720	0.80 (0.41-1.58)		
	No maternal supplementation + adequate VA dietary intake	19	487	22	506	0.90 (0.49-1.64)		
Low	No maternal supplementation + inadequate VA dietary intak	6	256	12	268	0.52 (0.20-1.37)		

[1] n is the number of deaths. N is the number of infants

[2] Vitamin A (VA). Adequate VA dietary intake is defined as <700 µg/day. Inadequate VA dietary intake is defined as 700 to <3000 µg/day.



Supplementary Figure 1A. Fixed-effects meta-analysis for the effect of neonatal vitamin A supplementation (NVAS) on infant mortality among infants born to HIV-infected women.

Title: Effect of delayed breastfeeding initiation on infant survival: a systematic review and meta-analysis

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ABSTRACT

Background: Early breastfeeding initiation is recommended, but not always considered an independent intervention to improve infant survival.

Methods: We conducted a systematic review and meta-analysis to assess the relationship between very early breastfeeding initiation (≤1 hour after birth) compared to delayed initiation (2-23 hours and ≥24 hours after birth) of breastfeeding on infant morbidity and mortality. We also assessed the association between early initiation (<24 hours of birth) compared to initiation 24 hours or more after birth on the same outcomes.

Results: We identified 4825 records after searching Pubmed, Embase, Web of Science, CINAHL, Popline, LILACS, AIM, and Index Medicus. We pooled five 'moderate quality' studies of 136,047 infants that examined the association between very early breastfeeding initiation and neonatal mortality. Compared to infants who initiated breastfeeding ≤1 hour after birth, infants who initiated breastfeeding 2-23 hours after birth had a 33% greater risk of neonatal mortality (95% CI: 13-56%, I^2=0%), and infants who initiated breastfeeding ≥24 hours after birth had a 2.19-fold greater risk of neonatal mortality (95% CI: 1.73-2.77, I^2=33%). Among the subgroup of infants exclusively breastfed in the neonatal period, those who initiated breastfeeding ≥24 hours after birth had an 85% greater risk of neonatal mortality compared to infants who initiated <24 hours after birth (95% CI: 29-167%, I^2=33 %).

Conclusions: Policy frameworks and models to estimate newborn and infant survival, as well as health facility policies, should consider the independent effect of early breastfeeding initiation.

Key Words: breastfeeding initiation, neonatal mortality, infant mortality, infant morbidity, infant nutrition

Key Messages

- Our review provides new insight on the increased risk of neonatal mortality associated with delayed breastfeeding initiation and demonstrated a clear dose-response relationship; the risk of neonatal mortality increased with increased delay in breastfeeding initiation.
- We found that delayed breastfeeding initiation was associated with a similar, increased risk of neonatal mortality among low birthweight infants and among those exclusively breastfeeding the neonatal period.
- We found limited evidence that delayed breastfeeding initiation is associated with increased risk of morbidity such as diarrhea, hypothermia, and umbilical cord infection. However, we could not produce pooled estimates due to differences in exposure and outcome definitions.
- We conclude that there is insufficient evidence regarding delayed breastfeeding initiation and nutritional status. Additional analyses utilizing data from high quality, prospective cohorts would strengthen the overall quality of the evidence.
- This review provides evidence that early breastfeeding initiation should be considered independently within policy frameworks or when models such as *LiST* estimate the survival benefits of breastfeeding. Breastfeeding promotion programs and health facility policies should also emphasize the importance of early initiation of breastfeeding, in addition to promoting exclusive breastfeeding. This is particularly relevant for countries, where neonatal and infant mortality rates are high, most women already exclusively or predominantly breastfeed their infants, and delay of initiation of breastfeeding beyond the first hour of life is common.

INTRODUCTION

Five million deaths in children younger than five years were reported globally in 2015; almost half (46%) of these occurred in the neonatal period [1]. An even a greater number of children are affected by prematurity, malnutrition, and septicaemia, which can result in serious physical and neurological sequelae [2]. Interventions that can be implemented at scale, starting before birth and continuing throughout the postnatal period, are needed to reduce mortality and morbidity in children and young infants [2]. Currently, only 50% of infants in the world are breastfed during the first hour of life, and 60% are exclusively breastfed [3]. The World Health Organization (WHO) recommends that newborns initiate breastfeeding within one hour of birth, but this recommendation is not supported by an official WHO guideline. Additional evidence is needed to inform public health investment and to facilitate the implementation of breastfeeding promotion programs.

Systematic reviews published in 2013 and 2015 reported that early breastfeeding initiation (defined in these reviews as initiation within 24 hours of birth) was associated with reduced neonatal mortality [4, 5]. However, no association was found in a subgroup analysis which examined risks among exclusively breastfed infants [4], and early breastfeeding initiation was not included as an independent intervention in the recent Lancet 2013 Nutrition Series [6]. Substantial data on the association between early breastfeeding initiation and neonatal mortality has recently become available [7-11], including new data from large cohorts of mothers and infants who participated in three neonatal vitamin A trials in Ghana, India, and Tanzania [12-14]. Data from these cohorts was used to examine the association between very early breastfeeding

initiation (defined as initiation within one hour of birth) and neonatal and post-neonatal mortality. These trials have also provided new data regarding early initiation among exclusively breastfed infants [15].

This paper reports the results of a systematic review of all studies published through December 2015 and updates pooled estimates of associations between delayed breastfeeding initiation and neonatal mortality. We assessed the relationship between very early initiation of breastfeeding (within one hour of birth) compared to delayed initiation (2-23 hours and 24 hours or more after birth) on neonatal mortality (<28 days). We also compared breastfeeding initiation within 24 hours of birth to initiation 24 hours or more after birth in order to update the results of the previous meta-analyses. We further examined the relationship between breastfeeding initiation time and infant morbidity and growth.

METHODS

Protocol and registration

The protocol for this review was developed by the co-authors after examining existing review articles. We registered the protocol with the International Prospective Register of Systematic Reviews (PROSPERO) (Registration Number CRD42015032321). We followed MOOSE Guidelines for the meta-analysis of observational data while conducting the search, analysis and writing the manuscript [16].

Inclusion and exclusion criteria

We included observational studies (*e.g.* cross-sectional studies, cohort studies, and case-control studies) and randomized control trials, if they examined the association between breastfeeding initiation time and mortality, morbidity, or nutrition outcomes from birth through 12 months of age in a population of infants who all initiated breastfeeding. There were no date restrictions. Studies were excluded if they were non-human studies, case reports or case study designs, or if the paper was published in abstract form only.

Definitions

The exposure of interest was breastfeeding initiation time. We assessed the relationship between very early initiation (within one hour of birth) compared to delayed initiation (2-23 hours and 24 hours or more after birth). We also compared breastfeeding initiation less than 24 hours to 24 hours or more.

Specific mortality outcomes of interest included: neonatal mortality (<28 days), infant mortality through six months (<180 days), and infant mortality through 12 months (<360 days). Specific morbidity and nutrition outcomes of interest included: diarrhea, respiratory infection, sepsis, omphalitis, hypothermia, weight loss, weight for age (WAZ), length-for- age (LAZ), weight-for-length (WLZ), and hospitalization.

Search

We conducted electronic searches from December 9-15, 2015. We searched Pubmed, Embase, Web of Science, CINAHL, Popline, LILACS, AIM, and Index Medicus for the Eastern Mediterranean Region. The search strategy included: (i) terms to identify papers regarding

breastfeeding, AND (ii) terms to identify papers regarding timing OR initiation, AND (iii) terms for mortality OR morbidity outcomes. There were no date restrictions. The full search strategy used for each database is available in Appendix 1.

Study selection, data collection, and quality assessment

Two reviewers independently assessed the titles and abstracts of all studies, removed duplicates, and categorized each paper as eligible, ineligible, or unclear using the eligibility criteria defined above. Disagreements were resolved through consultation with a third reviewer. Two reviewers independently extracted data for all studies that met the inclusion criteria including: study characteristics, study quality, and the effect estimates showing the relationship between breastfeeding initiation time and infant morbidity and mortality. When available, we used mortality estimates that excluded deaths in the first two to four days of life in order to rule out reverse causality. The quality of included studies was assessed using criteria developed in accordance with the World Health Organization (WHO) Child Health Epidemiology Reference Group (CHERG) (Table 2.1) and the overall quality of evidence was assessed using GRADE guidelines [17, 18].

Analyses

We pooled relative risks and 95% confidence intervals for all outcomes with two or more included studies. Because no heterogeneity was apparent, data synthesis was conducted using fixed effects meta-analysis. Heterogeneity of effects were assessed visually using Forest Plots of relative risks, quantified by the I^2, and tested by the Q statistic tests [19]. Q tests with p values

Table 2.1. Criteria used to classify the quality of included studies.

	Study Design	Selection Bias	Information Bias	Attrition bias	Confounding	Reverse Causality
High	RCT	Population-based recruitment	Assessed exposure within 30 days of birth and prior to outcome	Loss to follow up <10%	Model adjusts for gestational age or low birthweight. Other adjustments desirable.	Must exclude early infant deaths or those who unable to initiate breastfeeding early.
Moderate	-			Loss to follow up 10-<15%		
Low	Observational	Cross-sectional recruitment	Assessed exposure more than 30 days after birth or after outcome occurred	Loss to follow up 15-<20%	-	-
Very Low	-			Loss to follow up >20%		

<0.05 or I^2 values >50% were considered to represent substantial heterogeneity. All analyses were done using STATA 14 software.

We planned to use stratified meta-analyses to explore potential sources of heterogeneity on the association between breastfeeding initiation and infant mortality and morbidity. We defined the following subgroups *a priori*: study quality (comparing high quality studies to low and medium quality studies); low birthweight (<2500 g) compared to normal birthweight infants; exclusively breastfed in the neonatal period compared to not exclusively breastfed infants (including partial and predominant breastfeeding); high income countries (HIC) compared to low- and middle-income countries (LMIC) (as defined by the World Bank; and HIV-exposed infants compared to HIV-unexposed infants.

RESULTS

We found a total of 4825 records. After removing 1317 duplicates, we screened 3508 titles and abstracts. 184 papers were selected for full text screening (Figure 2.1). A total of 22 papers were eligible for inclusion in the analysis [7-11, 15, 20-35] (Appendix 2, Supplemental Table 2A). Three papers referred to the same study and study population [25-27], and they were subsequently considered as one study, "Edmond 2006". One paper contained pooled data from three studies [15], and we requested study-specific estimates from the authors so that each site could be included individually [12-14]. One study was categorised as high quality [35], seven studies were considered of moderate quality [9, 12-15, 27, 30-33], and 12 studies were

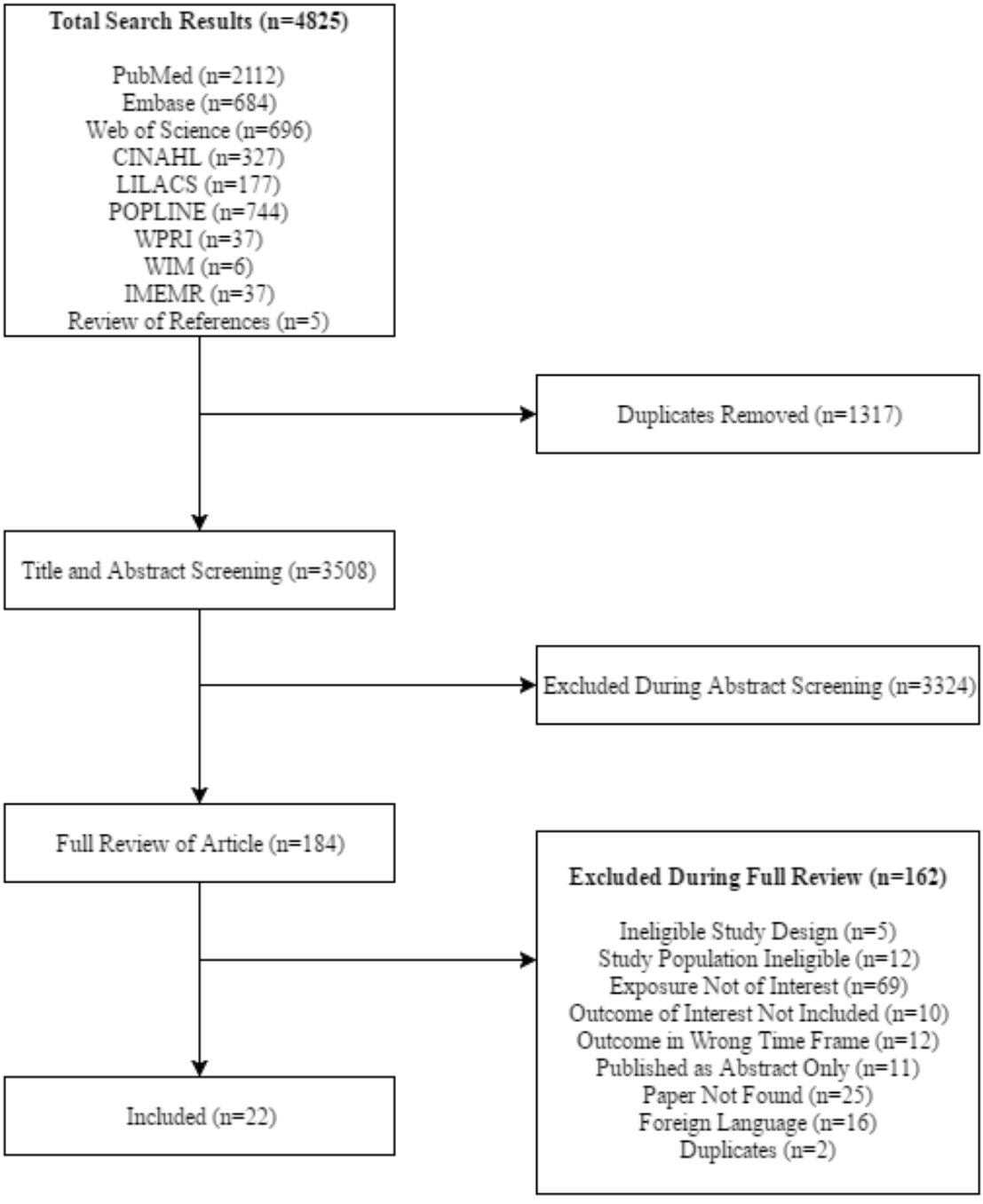

Figure 2.1. Search results

considered low or very low quality [7, 8, 10, 11, 20-24, 28, 29, 34] (Appendix 2, Supplemental Table 2B).

We identified 11 studies which examined breastfeeding initiation time and neonatal mortality [9, 10, 12-15, 20, 21, 27, 30, 33, 34] (Table 2.2). We pooled five of these studies which examined the association between delayed breastfeeding initiation (2-23 hours or ≥24 hours) compared to very early initiation (≤1 hour) on neonatal mortality, including a total of 136,047 infants [12-15, 27, 33]. There was evidence of a dose response relationship; increasing delay in breastfeeding initiation time was associated with an increasing risk of neonatal mortality. Infants who initiated breastfeeding 2-23 hours after birth had a 33% greater risk of neonatal mortality (95% CI: 13-56%), and infants who initiated breastfeeding ≥24 hours after birth were more than twice as likely to die during the neonatal period (pooled RR 2.19, 95% CI: 1.73-2.77) when compared to those who initiated breastfeeding within one hour of birth. There was no evidence of heterogeneity of effect (Figure 2.2). All pooled studies were categorised as 'moderate' quality. In a sensitivity analysis, we included estimates from Garcia *et al.* 2011 [30], which defined 'early initiation' as breastfeeding initiation <12 hours, and we found similar results (Appendix 2 - Figure 2A).

Six studies examined the association between breastfeeding initiation within 24 hours compared to ≥24 hours on neonatal mortality, including a total of 142,729 infants [12-15, 27, 30, 33]. In the largest cohorts relatively few infants initiated breastfeeding after 24 hours: 302 infants in Ghana, 236 infants in Tanzania, and 4,039 infants in India. Infants who initiated breastfeeding more than 24 hours after birth had a 70% greater risk of neonatal mortality compared to infants who

Table 2.2. Summary of studies of the association between early breastfeeding initiation and neonatal mortality. (*Reference group)

Study	N	Study Design	Exposure Definition	Effect Estimate (95% CI)	Quality
Neovita (India)	44,984	Prospective Cohort	Early (<1 hr)* vs. Late (2-23, ≥24 hrs) breastfeeding initiation	aRR(2-23 hrs): 1.18 (0.93-1.49) aRR(≥24 hrs): 1.61 (1.11-2.35)	Moderate
Neovita (Ghana)	22,955	Prospective Cohort	Early (<1 hr)* vs. Late (2-23, ≥24 hrs) breastfeeding initiation	aRR(2-23 hrs): 1.41 (1.01-1.98) aRR(≥24 hrs): 3.68 (1.82-7.46)	Moderate
Neovita (Tanzania)	31,999	Prospective Cohort	Early (<1 hr)* vs. Late (2-23, ≥24 hrs) breastfeeding initiation	aRR(2-23 hrs): 1.61 (1.06-2.44) aRR(≥24 hrs): 1.90 (0.47-7.62)	Moderate
Akter 2015	3,190	Cross-sectional	Early (<1 hr) vs. Late* (>1 hr) breastfeeding initiation	aOR: 0.86 (0.41-1.82)	Very Low
Shah 2014	6,399	Prospective Cohort. Preterm infants only.	Early (<1 hr) vs. Late* (>1 hr) breastfeeding initiation	aRR: 0.7 (: 0.6-1.0)	Moderate
Sutan 2014	500	Case Control. Low birthweight infants only.	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	aOR: 2.03 (: 1.09-3.90)	Very Low
Niswade 2011	1087	Prospective Cohort. Tribal infants only.	Early* vs. Late breastfeeding initiation	aOR (tribal): 3.1 (05% CI:0.9-10.1)	Very Low
Garcia 2011	10,352	Prospective Cohort	Early (<12 hr)* vs. Late (12-23, ≥24 hrs) breastfeeding initiation	aRR(12-24 hrs): 0.93 (: 0.59-1.46) aRR (>24 hrs): 1.76 (: 1.01-3.07)	Moderate
Edmond 2006	10,942	Prospective Cohort	Early (<1 hr)* vs. Late (2-23 hrs, Day 2, Day 3, ≥Day 4) breastfeeding initiation	aOR(Day1): 1.45 (0.90-2.35) aOR(Day2): 2.70 (1.70-4.3) aOR(Day3): 3.01 (1.70-5.38) aOR(≥Day4): 4.42 (1.76-11.09)	Moderate
Bamji 2008	4,357	Case Control	Early (Day 1) vs. Late (Day 2, ≥Day 3) breastfeeding initiation	OR(Day2): 1.58 (0.17-14.51) OR(≥Day3): 10.14 (3.17-32.42)	Very Low
Mullany 2008	22,838	Prospective Cohort	Early (<1 hr)* vs. Late (2-23 hrs, Day 2, Day 3, ≥Day 4) breastfeeding initiation	aOR(Day1): 1.43 (0.52-3.89) aOR(Day2): 1.78 (0.64-5.00) aOR(Day3): 2.43 (0.86-6.90) aOR(>Day4): 2.06 (0.62-6.82)	Moderate

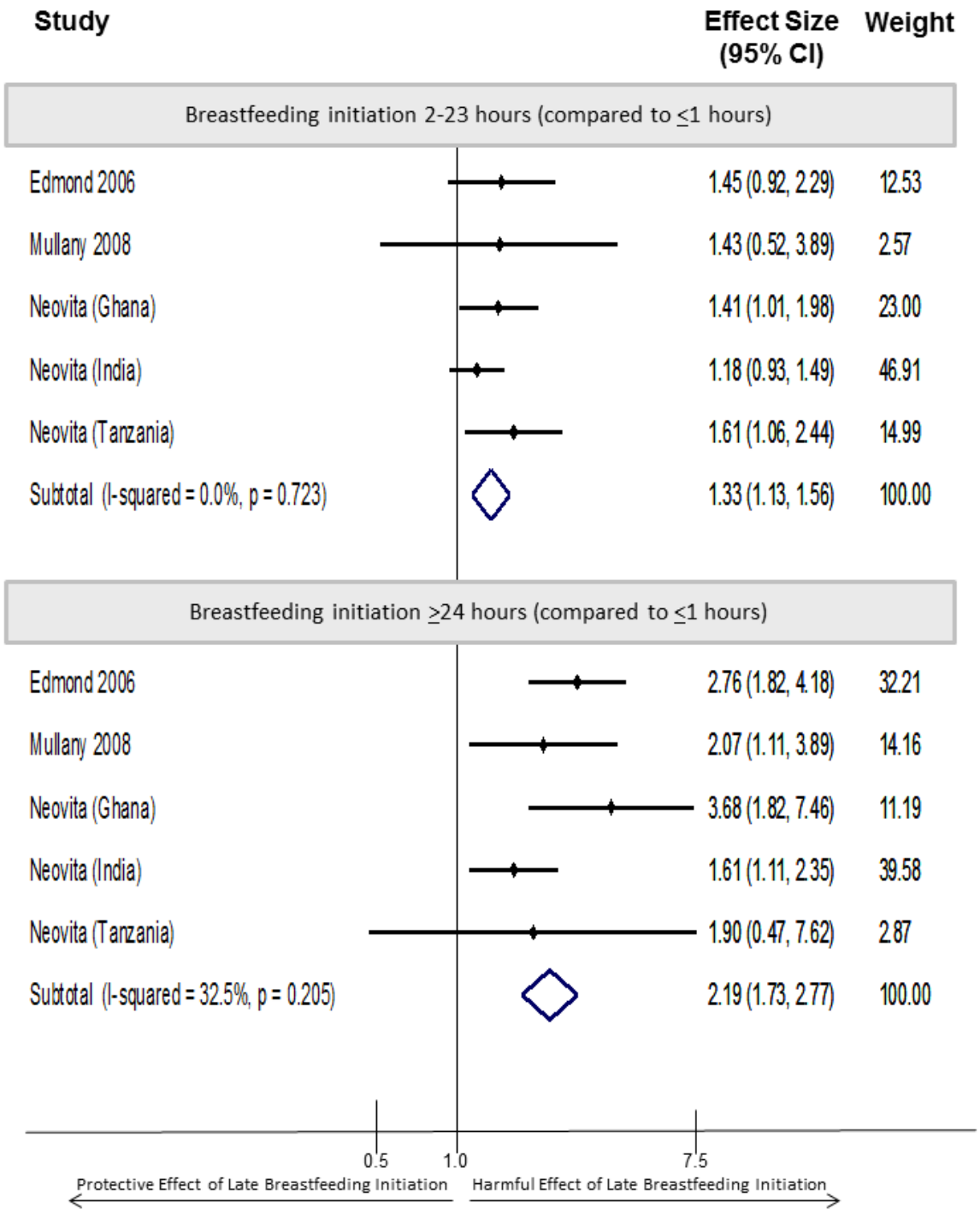

Figure 2.2. Forest Plot of the relative risk of neonatal mortality (excluding deaths in the first 2-4 days) for infants who initiated breastfeeding 2-23 hours or ≥24 hours after birth, compared to those who initiated breastfeeding early (≤1 hour).

initiated breastfeeding within 24 hours after birth (pooled RR 1.70, 95% CI: 1.44-2.01) (Figure 2.3). There was no evidence of substantial heterogeneity of effect (X^2 p value=0.13, I^2=41 %).

It was only possible to examine the association between very early initiation (<1 hour) and neonatal mortality among exclusively breastfed infants in three studies [12-14], and pooled estimates including these three cohorts have previously been published [15]. However, there were five studies which examined the relationship between breastfeeding initiation ≥24 hours compared to <24 hours and neonatal mortality among exclusively breastfed infants. The effect size was incalculable in the large India site [12], as there were no deaths among the small group of infants initiating breastfeeding ≥24 hours (n=150). Thus, we pooled the estimates from four studies, including a total of 65,215 infants [13-15, 27, 33]. Infants who were exclusively breastfed in the neonatal period who delayed breastfeeding initiation 24 hours or more after birth had an 85% increased risk of neonatal mortality compared to infants who initiated breastfeeding early (<24 hours after birth) (pooled RR 1.85, 95% CI: 1.29-2.67) (Figure 2.3). There was no evidence of heterogeneity of effect (X^2 p value=0.21, I^2=33%).

The same five studies included data which allowed examination of the relationship between delayed breastfeeding initiation (≥24 hours) compared to <24 hours and neonatal mortality among low birth weight infants. However, the effect was incalculable in the Tanzania site [14] as there were no deaths among the small group of low birthweight infants initiating breastfeeding after 24 hours (n=35). Thus, we pooled the estimates from four studies, including a total of 21,258 infants [12, 13, 15, 27, 33]. Low birthweight infants who initiated breastfeeding more than 24 hours after birth had a 73% greater risk of neonatal mortality compared to infants who

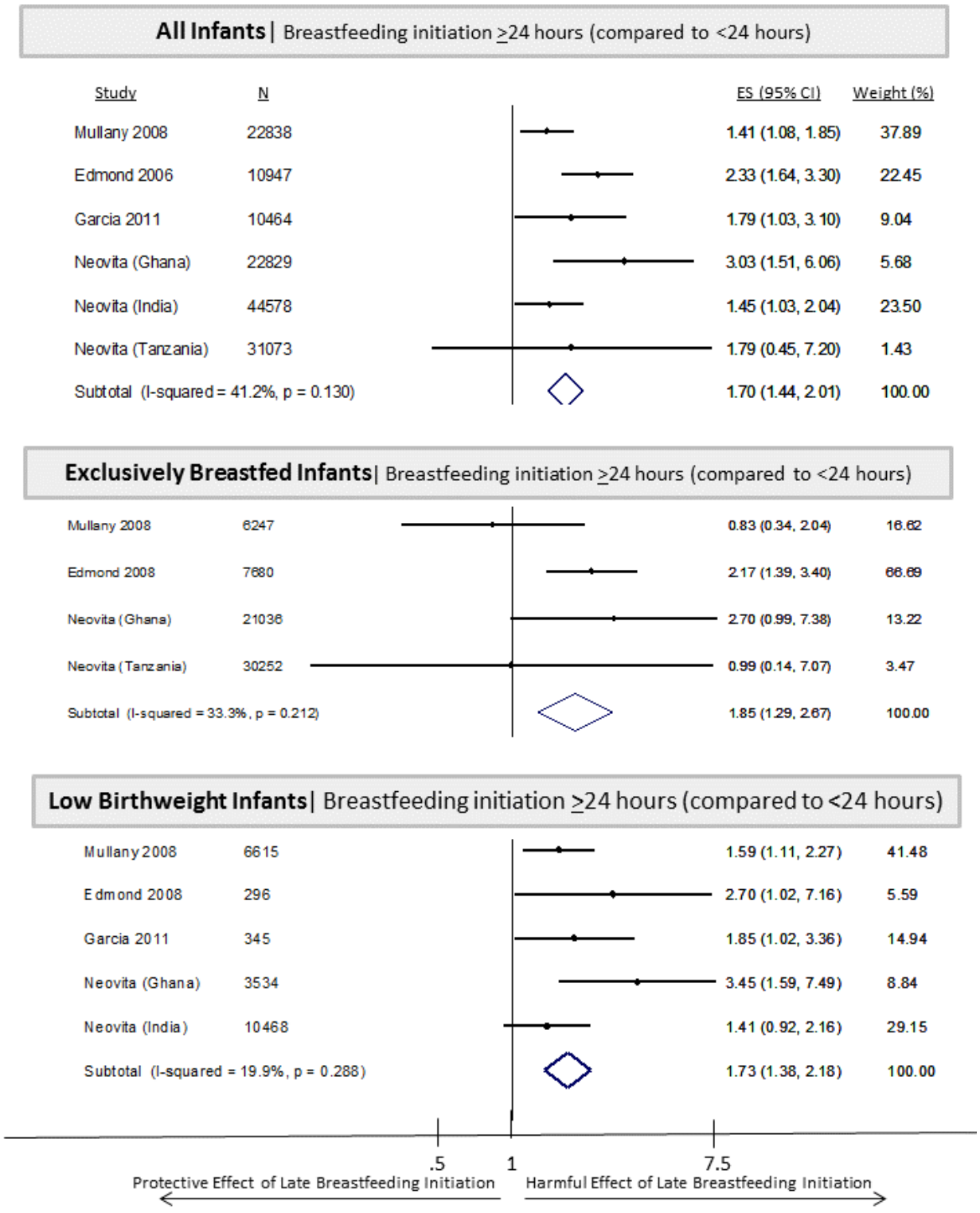

Figure 2.3. Forest Plot of the relative risk of neonatal mortality (excluding deaths in the first 2-4 days) for infants who initiated breastfeeding ≥24 hours after birth, compared to those who initiated breastfeeding early (<24 hours) for i) all infants, ii) among exclusively breastfed infants, iii) among low birthweight infants.

initiated breastfeeding <24 hours after birth (pooled RR 1.73, 95%CI: 1.38-2.18) (Figure 2.3). There was no evidence of heterogeneity of effect (X^2 p value=0.29, I^2=20%).

We were unable to perform other subgroup analyses (*e.g.* by quality score; high-, middle-, or low-income status of country; maternal HIV status; etc.) as there were less than two studies in each subgroup strata. Only one study (including the three neonatal vitamin A trial cohorts) presented effect estimates for early infant mortality (one to three months and three to six months) [15], and no studies presented effect estimates for infant mortality through 12 months.

Table 2.3 summarizes the combined evidence regarding the association between delayed breastfeeding initiation and neonatal mortality. The overall quality of the evidence illustrating an increased risk of death among infants that initiate breastfeeding more than one hour after birth is rated as "high" quality. Although the pooled effect size is based on observational studies, the quality rating was upgraded because there is an apparent dose response relationship and a large increased risk of death (RR >2.0) for infants initiating ≥24 hours after birth. The other analyses that compared the risk of neonatal mortality for those initiationg breastfeeding ≥24 hours after birth to those initiating <24 hours after birth among all infants, exclusively breastfed infants, and low birthweight infants, were classified as "moderate" in overall quality. The evidence is based on high-quality observational studies (which are considered to be "moderate" in quality due to the inherent limitations of observational studies), and the quality was not upgraded because a potential dose response relationship is not examined and the pooled relative risks are less than two.

Table 2.3. Summary of findings regarding the association between delayed breastfeeding association and neonatal mortality

Outcome	Population	Illustrative comparative risks (95% CI) Assumed risk[1] Early Breastfeeding	Illustrative comparative risks (95% CI) Corresponding risk[2] Delayed Breastfeeding	Relative effect (95% CI)	Number of participants (studies)	Quality of the evidence (GRADE)[3]
Neonatal Mortality	All infants, who ever initiated breastfeeding, surviving 2-4 days	<1 Hour: 5.2 per 1000	2-23 Hours: 6.9 per 1000 (5.9 to 8.1) ≥24 Hours: 11.4 per 1000 (9.0 to 14.4)	(2-23 Hours): 1.33 (1.13-1.56) (≥24 Hours): 2.19 (1.73-2.77)	136,047 (5 studies)	High[4]
	All infants, who ever initiated breastfeeding, surviving 2-4 days	<24 Hours: 7.7 per 1000	≥24 Hours: 13.1 per 1000 (11.1 to 15.5)	1.70 (1.44-2.01)	142,729 (6 studies)	Moderate[5]
	Exclusively breastfeeding infants, who ever initiated breastfeeding, surviving 2-4 days	<24 Hours: 6.9 per 1000	≥24 Hours: 12.4 per 1000 (8.9 to 18.4)	1.85 (1.29-2.67)	65,215 (4 studies)	Moderate[5]
	Low birthweight infants, who ever initiated breastfeeding, surviving 2-4 days	<24 Hours: 18.0 per 1000	≥24 Hours: 31.1 per 1000 (24.8 to 39.2)	1.73 (1.38-2.18)	21,258 (4 studies)	Moderate[5]

[1] The assumed risk is the median risk in the 'early breastfeeding' group across all studies providing this information.

[2] The corresponding risk is based on the assumed risk in the 'early breastfeeding' group and the relative effect of the intervention (and its 95% confidence interval).

[3]GRADE Working Group grades of evidence description [17]: High quality: Further research is very unlikely to change our confidence in the estimate of effect.; Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.; Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.; Very low quality: We are very uncertain about the estimate.

[4] All five studies are categorized as having a moderate risk of bias, but the overall strength of evidence is upgrade to 'High' because the studies are consistent, there is a large effect size (RR >2), and there is evidence dose response.

[5] All studies are categorized as having a moderate risk of bias. There is no evidence of dose response (due to study design) and there is no large effect size.

All six studies of timing of breastfeeding initiation and nutritional status (*e.g.* stunting, wasting, underweight, or early weight loss) were considered very low quality. We could not pool any of the estimates due to variations in the exposure definition or the time period of outcome assessment (Table 2.4). One cross-sectional study from Guatemala observed 67% increased risk of stunting and more than threefold increased risk of being underweight among infants age <46 days who initiated breastfeeding after one hour of birth compared to infants who initiated breastfeeding within one hour of birth [11]. Two other studies found no relationship between breastfeeding initiation and the risk of stunting or underweight [8, 28]. None of the three cross-sectional studies which assessed wasting found a relationship with breastfeeding initiation time [8, 11, 28]. Similarly, there was no observed relationship between breastfeeding initiation time and early weight loss in three studies [22, 24, 29].

There were five studies which examined the relationship between timing of breastfeeding initiation on morbidity (*e.g.* diarrhea, respiratory infections, hypothermia, and umbilical cord infection) with mixed quality levels (Table 2.5). We were unable to pool any of the morbidity studies due to differences in exposure definition, time of outcome assessment, or difference in type of effect estimate (Table 2.5). Two studies showed a reduced risk of diarrhea among infants aged <6 months associated with early breastfeeding initiation (within three days after birth [23] or within one hour [7]). Mullany and colleagues reported an association between delayed breastfeeding and an increased risk of umbilical cord infection among infants in Tanzania [31] and an increased risk of hypothermia among infants in Nepal [32]. Similarly, Van den Bosch reported a two-fold greater risk of hypothermia among infants randomized to "mother's choice

Table 2.4. Summary of studies of the association between early breastfeeding initiation and nutrition outcomes. (*Reference group)

Study	Sample Size	Study Design	Exposure Definition	Outcome Definition	Effect Estimate	Quality
Malnutrition (Stunting / HAZ)						
Wren 2015	190	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	HAZ < –2 SD at <46 days	RR: 1.67 (95% CI: 1.15-2.43)	Very Low
Meshram 2012	351	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	HAZ < –2 SD at 1 year	RR: 1.19 (95% CI: 0.63-2.26)	Very Low
Engebretsen 2008	723	Cross-Sectional	Early (<2 hrs)* vs. Late (2-24 hrs, >24 hrs) breastfeeding initiation	HAZ < –2 SD at <1 year	OR: (2-24 hrs): 1.06 (95% CI: 0.56-1.99) OR: (>24 hrs): 1.27 (95% CI: 0.80-2.03)	Very Low
Malnutrition (Wasting / WHZ)						
Wren 2015	190	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	WHZ < –2 SD at <46 days	RR: 0.84 (95% CI: 0.14-4.88)	Very Low
Meshram 2012	351	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	WHZ < –2 SD at 1 year	RR: 0.85 (95% CI: 0.52-1.38)	Very Low
Engebretsen 2008	723	Cross-Sectional	Early (<2 hrs)* vs. Late (2-24 hrs, >24 hrs) breastfeeding initiation	WHZ < –2 SD at <1 year	OR: (2-24 hrs): 1.07 (95% CI: 0.39-2.92) OR: (>24 hrs): 0.81 (95% CI: 0.33-2.06)	Very Low
Malnutrition (Underweight / WAZ)						
Wren 2015	190	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	WAZ < –2 SD at <46 days	RR: 3.06 (95% CI: 1.49-6.29)	Very Low
Meshram 2012	351	Cross-Sectional	Early (<1 hr)* vs. Late (>1 hr) breastfeeding initiation	WAZ < –2 SD at 1 year	RR: 0.87 (95% CI: 0.59-1.28)	Very Low
Malnutrition (Early weight loss)						
Dewey 2003	280	Prospective Cohort	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Weight loss >10% since birth at 3 days	RR: 1.20 (95% CI: 0.62-2.33)	Very Low
Caglar 2006	90	Case-Control	Mean time to first breastfeeding (hrs)	Weight loss ≥10% since birth at 4-9 days	Weight loss ≥10% group: 3.89 hrs (SE 2.37) Weight loss <10% group: 2.14 hrs (SE 1.31)	Very Low
Enzunga 1990	330	Prospective Cohort	Time of breastfeeding initiation (hrs)	Weight loss since birth	Time of breastfeeding initiation and weight loss were associated in univariate and multiavriate linear regression	Very Low

Table 2.5. Summary of studies of the association between early breastfeeding initiation and morbidity outcomes (*Reference group)

Study	Sample Size	Study Design	Exposure Definition	Outcome Definition	Effect Estimate	Quality
Diarrhea						
Clemmens 1999	198	Prospective Cohort	Early (<3 days) vs. Late (≥3 days)* breastfeeding initiation	Diarrhea at <6 months	aRR: 0.74 (95% CI: 0.56-0.98)	Low
Clemmens 1999	198	Prospective Cohort	Early (<3 days) vs. Late (≥3 days)* breastfeeding initiation	Diarrhea at 6-12 months	aRR: 0.95 (95% CI: 0.70-1.31)	Low
Hajeebhoy 2014	6068	Cross-Sectional	Early (<1 hr) vs. Late (>1 hr)* breastfeeding initiation	Diarrhea at <6 months	aOR: 0.74 (95% CI:0.58-0.93)	Very low
ARI						
Hajeebhoy 2014	6068	Cross-Sectional	Early (<1 hr) vs. Late (>1 hr)* breastfeeding initiation	ARI at <6 months	aRR: 0.91 (95% CI: 0.80-1.03)	Very low
Hypothermia						
Mullany 2010	19180	Prospective Cohort	Early (<24 hrs)* vs. Late (>24 hrs) breastfeeding initiation	Prevalence of axillary measures <35.0°C at <28 days	aRR: 1.19 (95% CI: 1.08-1.30)	Moderate
Van den Bosch 1990	160	Randomized Trial	Immediate* vs. Mother's choice of breastfeeding initiation time.	Rectal temperatue <36.5°C at 2, 4, and ~24 hrs after birth	RR: 2.45 (95% CI: 1.36-4.41)	High
Umbilical Cord Infection						
Mullany 2009	1653	Prospective Cohort	Early (<1 hr) vs. Late (≥1 hr)* breastfeeding initiation	1. Pus with any redness (Broad) 2. Moderate or severe redness (Restrictive)	1. aRR: 0.74 (95% CI: 0.38-1.47) 2. aRR: 0.29 (95% CI: 0.11-0.74)	Moderate

of breastfeeding initiation time" compared to those randomized to immediate breastfeeding initiation in Malawi [35].

DISCUSSION

Our review provides new insight on the increased risk of neonatal mortality associated with delayed breastfeeding initiation (defined in this review as initiation after the first hour after birth). We demonstrated a clear dose-response relationship; the risk of neonatal mortality increased with increased delay in breastfeeding initiation. Infants who initiated breastfeeding between 2-23 hours after birth had a 33% greater risk of neonatal mortality compared to infants who initiated breastfeeding within an hour of birth. Neonatal mortality risk was more than 100% greater in infants who initiated breastfeeding more than 24 hours after birth. Our findings are based on five prospective cohort studies of 136,047 breastfed, live born infants who survived the first two to four days of life.

The intervention of interest (*i.e.* early breastfeeding initiation) has been inconsistently defined across studies. Some authors define "early breastfeeding initiation" as breastfeeding within one hour of birth (as we do here); others define early initiation as "within three days of birth" [23]. Two previously published meta-analyses defined early breastfeeding initiation as initiation within 24 hours of birth [4, 5]. However, in our three largest cohorts, accounting for nearly 100,000 infants [12-15], very few mothers initiated breastfeeding after 24 hours (n=4,577). In addition, the World Health Organization (WHO) and the United Nations Children's Fund

[UNICEF] recommend that breastfeeding is initiated within an hour of birth. Thus, we proposed that the primary intervention of interest for this study should be the effect of breastfeeding initiation within an hour of birth (*i.e.* very early breastfeeding initiation). Using this definition of "very early breastfeeding initiation", we pooled effect estimates for all-cause neonatal mortality for more than 136,000 infants enrolled in prospective cohorts in Ghana, India, Nepal, and Tanzania [12-15, 27, 33]. Similar effects were demonstrated after pooling the six studies [12-15, 27, 30, 33] which assessed the effect of breastfeeding initiation <24 hours. We found that early initiation of breastfeeding was beneficial, with a very similar effect size, even when the analysis was restricted to low birth weight infants. Due to the higher baseline risk of death among low birthweight infants, large gains in the number of deaths averted may be achieved through very early breastfeeding initiation in this group.

We also found that early initiation of breastfeeding was similarly beneficial, even when the analysis was restricted to exclusively breastfed infants. We have previously reported a similar finding regarding the effect of breastfeeding initiation within an hour of birth on neonatal mortality in exclusively breastfed infants using pooled data from nearly 100,000 infants enrolled in neonatal vitamin A supplementation trials [15]. This pooled analysis demonstrated a strong protective effect of very early breastfeeding initiation (within one hour of life) among infants who were exclusively breastfed in the neonatal period, at one month, and at three months of life [15]. This systematic review and meta-analysis found there was no additional data to pool beyond that provided by the neonatal vitamin A supplementation trials, as no other studies examined the effect of very early breastfeeding (within one hour) in exclusively breastfed infants. However, we updated the meta-analysis regarding early initiation of breastfeeding

(within 24 hours) among exclusively breastfed infants, though we were unable to include the largest cohort (Mazunder 2015) as the number of exclusively breastfed infants who initiated breastfeeding more than 24 hours after birth was very small. The pooled results of four studies (including 65,215 infants) showed that initiation of breastfeeding after the first 24 hours of life was associated with an 85% increased risk of neonatal mortality compared to infants who initiated breastfeeding within 24 hours after birth, and there was no evidence of heterogeneity of effect. We previously postulated that early initiation of breastfeeding independently reduces neonatal and early infant mortality by specific biological mechanisms, in addition to increasing rates of exclusive breastfeeding [15]. Our new meta-analysis provides additional evidence to support this hypothesis. This is in contrast to the previous meta-analysis which reported that there was no association between early breastfeeding (within 24 hours) and all-cause neonatal mortality among those that were exclusively breastfed [4]. However, as noted by Debes *et al*, there was limited data available to examine the exclusively breastfed subgroup at that time.

We identified five studies which examined the effect of early breastfeeding initiation on morbidity (*e.g.* diarrhea, respiratory infections, hypothermia, and umbilical cord infection) [7, 23, 31, 32, 35], and there were six studies that examined nutrition outcomes [8, 11, 22, 24, 28, 29]. However, most papers had a 'low' or 'very low' quality score, and we were unable to pool the study-specific estimates due to differences in the exposure definition, time of outcome assessment, or type of published effect estimate. Additional, higher quality research is needed to understand the relationship between early breastfeeding initiation and infant morbidity and nutrition outcomes.

Our findings have important implications for prioritizing interventions to improve neonatal survival. There is a strong biological basis for the survival benefits associated with early breastfeeding. Early breastfeeding initiation exposes the infant to maternal colostrum, which is thought to decrease the risk of microbial translocation, accelerate intestinal maturation, and promote resistance and epithelial recovery from infection [7-9]. Early breastfeeding may also reduce hypothermia and foster attachment and bonding through close contact with the mother [9]. Based on our review of the evidence, we recommend that early initiation of breastfeeding should be considered when estimating the overall survival benefits of breastfeeding, and should be considered for inclusion in models that assess the benefits of interventions for infant survival, such as those used in the Lives Saved Tool (LiST) [18].

There are several strengths of this systematic review and meta-analysis. First, we conducted a thorough review of the literature using all appropriate search engines, without limitations based on date of publication. For all outcomes, we provided a narrative synthesis of the evidence to account for the heterogeneity of exposure definition. Finally, the meta-analysis of the effect of breastfeeding initiation and all-cause infant mortality is based on large cohorts nested within well-conducted, population-based, randomized control trials, and we analysed the data to demonstrate the strength of a dose-response relationship. Our review and meta-analysis also had several limitations. We were unable to perform subgroup analyses by quality score, income status of country, or maternal HIV status as there were insufficient studies in these subgroups. Three studies [12-14], which have previously published pooled estimates [15], presented effect estimates for infant mortality between one to three months and three to six months, and no studies presented effect estimates for infant mortality through 12 months. The review was also

based entirely on observational data. However, a randomized trial of this intervention would not be considered ethical, so we must rely on methodologically robust analysis of high quality observational data. Additional research among high quality, prospective cohorts regarding the relationship between early breastfeeding initiation and cause-specific mortality and severe morbidity would strengthen the overall quality of the evidence.

Our study suggests that the effects of early breastfeeding initiation should be taken into account when policy frameworks or models such as *LiST* are applied to estimate the survival benefits of breastfeeding. Breastfeeding promotion programs which can remove structural, cultural, and information barriers to promote breastfeeding should also emphasize the importance of early initiation of breastfeeding, in addition to promoting exclusive breastfeeding. Furthermore, health facility policies and health provider knowledge can promote early breastfeeding initiation. This is particularly relevant for countries, where neonatal and infant mortality rates are high, most women already exclusively or predominantly breastfeed their infants, and delayed initiation of breastfeeding beyond the first hour of life is common.

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infancy in Ghana (Neovita): a randomised, double-blind, placebo-controlled trial. Lancet, 2015. **385**(9975): p. 1315-23.

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Appendix 1
Specific Search Strategy

Database: PubMed
Date of Search: 2015-12-09
Results: 2112 Records
Search Strategy:
("Breast Feeding"[Mesh] OR breast fe*[tiab] OR breastfe*[tiab])
AND
("Time Factors"[mesh] OR initiat*[tiab] OR timing[tiab] OR delay*[tiab] OR start[tiab] OR starting[tiab] OR starts[tiab] OR started[tiab] OR early breast* OR early suckl*[tiab])
AND
("Infant Mortality"[Mesh] OR "Infant Death"[Mesh] OR "Perinatal Death"[Mesh] OR "mortality"[Subheading] OR "Survival"[Mesh] OR mortality[tiab] OR death*[tiab] OR died[tiab] OR survival[tiab] OR "Diarrhea"[Mesh] OR diarrhea*[tiab] OR diarrhoea*[tiab] OR "Respiratory Tract Infections"[Mesh] OR respiratory infect*[tiab] OR respiratory illness*[tiab] OR respiratory disease*[tiab] OR respiratory tract infect*[tiab] OR influenza[tiab] OR pneumonia[tiab] OR bronchitis[tiab] OR "Sepsis"[Mesh] OR sepsis[tiab] OR septic[tiab] OR bacteremia[tiab] OR septicemi*[tiab] OR ("Umbilical Cord"[mesh] AND "Bacterial Infections and Mycoses"[Mesh]) OR omphalitis[tiab] OR umbilical cord infection*[tiab] OR "Hospitalization"[Mesh] OR hospitali*[tiab] OR hospital admission*[tiab] OR "Malnutrition"[Mesh] OR "Infant Nutrition Disorders"[Mesh] OR "Child Nutrition Disorders"[Mesh] OR "Wasting Syndrome"[Mesh:noexp] OR "Weight Gain"[mesh] OR "Weight Loss"[mesh] OR malnutri*[tiab] OR malnourish*[tiab] OR mal nutri*[tiab] OR under nourish*[tiab] OR undernutrition[tiab] OR under nutrition[tiab] OR nutritional status[tiab] OR weight gain[tiab] OR weight loss[tiab] OR underweight[tiab] OR under weight[tiab] OR growth failure[tiab] OR marasmus[tiab] OR kwashiorkor[tiab] OR stunt*[tiab] OR wasting[tiab] OR weight for age[tiab] OR length for age[tiab] OR weight for length[tiab])
AND
("Infant"[Mesh] OR "Child, Preschool"[Mesh] OR infant*[tiab] OR new born*[tiab] OR newborn*[tiab] OR neonat*[tiab] OR baby[tiab] OR babies[tiab] OR child*[tiab])

Database: Embase
Date of Search: 2015-12-09
Results: 684 Records
Search Strategy:
(('breast feeding'/exp AND 'time'/exp) OR (('breast fed' OR 'breast feed' OR 'breast feeds' OR 'breast feeding' OR breastfe* OR suckl*) NEAR/3 (initiat* OR timing OR delay* OR start OR starting OR starts OR started OR early OR immediate OR beginning)):ab,ti)
AND
('infant mortality'/exp OR 'newborn death'/exp OR 'perinatal death'/exp OR 'perinatal mortality'/exp OR 'survival'/exp OR mortality:ab,ti OR death*:ab,ti OR died:ab,ti OR survival:ab,ti OR 'infantile diarrhea'/exp OR diarrhea*:ab,ti OR diarrhoea*:ab,ti OR 'respiratory tract infection'/exp OR (respiratory NEAR/3 (infect* OR illness* OR disease*)):ab,ti OR influenza:ab,ti OR pneumonia:ab,ti OR bronchitis:ab,ti OR 'newborn sepsis'/exp OR sepsis:ab,ti OR septic:ab,ti OR bacteremia:ab,ti OR septicemi*:ab,ti OR 'omphalitis'/exp OR omphalitis:ab,ti OR (('umbilical cord' OR umbilicus) NEAR/3 infection*):ab,ti OR 'child hospitalization'/exp OR hospitali*:ab,ti OR (hospital NEAR/3 admission*):ab,ti OR 'malnutrition'/exp OR 'nutritional deficiency'/exp OR 'wasting syndrome'/exp OR 'weight gain'/exp OR 'weight loss'/exp OR malnutri*:ab,ti OR malnourish*:ab,ti OR undernourish*:ab,ti OR (mal NEXT/1 nutri*):ab,ti OR (under NEXT/1 nourish*):ab,ti OR undernutrition:ab,ti OR 'under nutrition':ab,ti OR 'nutritional status':ab,ti OR 'weight gain':ab,ti OR 'weight loss':ab,ti OR

underweight:ab,ti OR 'under weight':ab,ti OR 'growth failure':ab,ti OR marasmus:ab,ti OR kwashiorkor:ab,ti OR stunt*:ab,ti OR wasting:ab,ti OR 'weight for age':ab,ti OR 'length for age':ab,ti OR 'weight for length':ab,ti)
AND
('infant'/exp OR 'preschool child'/exp OR infant*:ab,ti OR (new NEXT/1 born*):ab,ti OR newborn*:ab,ti OR neonat*:ab,ti OR baby:ab,ti OR babies:ab,ti OR child*:ab,ti)

Database: Web of Science
Science Citation Index Expanded (SCI-EXPANDED) --1900-present
Social Sciences Citation Index (SSCI) --1900-present
Conference Proceedings Citation Index- Science (CPCI-S) --1990-present
Conference Proceedings Citation Index- Social Science & Humanities (CPCI-SSH) --1990-present
Date of Search: 2015-12-10
Results: 696 Records
Search Strategy:
TS=(("breast fed" OR "breast feed" OR "breast feeds" OR "breast feeding" OR "breastfe*" OR "suckl*") NEAR/3 ("initiat*" OR "timing" OR "delay*" OR "start" OR "starting" OR "starts" OR "started" OR "early" OR "immediate" OR "beginning"))
AND
TS=("mortality" OR "death*" OR "died" OR "survival" OR "diarrhea*" OR "diarrhoea*" OR ("respiratory" NEAR/3 ("infect*" OR "illness*" OR "disease*")) OR "influenza" OR "pneumonia" OR "bronchitis" OR "sepsis" OR "septic" OR "bacteremia" OR "septicemi*" OR "omphalitis" OR (("umbilical cord" OR "umbilicus") NEAR/3 infection*) OR "hospitali*" OR ("hospital" NEAR/3 "admission*") OR "malnutri*" OR "undernourish*" OR "mal nutri*" OR "malnourish*" OR "under nourish*" OR "undernutrition" OR "under nutrition" OR "nutritional status" OR "weight gain" OR "weight loss" OR "underweight" OR "under weight" OR "growth failure" OR "marasmus" OR "kwashiorkor" OR "stunt*" OR "wasting" OR "weight for age" OR "length for age" OR "weight for length")
AND
TS=("infant*" OR "new born*" OR "newborn*" OR "neonat*" OR "baby" OR "babies" OR "child*")

Database: CINAHL
Date of Search: 2015-12-10
Results: 327 Records
Search Strategy:
(MH ("Time Factors" AND MH "Breast Feeding+") OR TI (("breast fed" OR "breast feed" OR "breast feeds" OR "breast feeding" OR breastfe* OR suckl*) N3 (initiat* OR timing OR delay* OR start OR starting OR starts OR started OR early OR immediate OR beginning)) OR AB (("breast fed" OR "breast feed" OR "breast feeds" OR "breast feeding" OR breastfe* OR suckl*) N3 (initiat* OR timing OR delay* OR start OR starting OR starts OR started OR early OR immediate OR beginning)))
AND
(MH ("Infant Mortality" OR "Infant Death" OR "Perinatal Death" OR "Diarrhea") OR "Diarrhea (NANDA)" OR "Respiratory Tract Infections+" OR "Sepsis+" OR "Hospitalization" OR "Malnutrition" OR "Infant Nutrition Disorders" OR "Child Nutrition Disorders" OR "Nutitional Status" OR "Nutritional Status (Iowa NOC)" OR "Wasting Syndrome" OR "Weight Gain" OR "Weight Loss") OR TI ("mortality" OR "death*" OR "died" OR "survival" OR "diarrhea*" OR "diarrhoea*" OR ("respiratory" N3 ("infect*" OR "illness*" OR "disease*")) OR "influenza" OR "pneumonia" OR "bronchitis" OR "sepsis" OR "septic" OR "bacteremia" OR "septicemi*" OR "omphalitis" OR (("umbilical cord" OR "umbilicus") N3 infection*) OR "hospitali*" OR ("hospital" N3 "admission*") OR "malnutri*" OR "malnourish*" OR "undernourish*" OR "mal nutri*" OR "under nourish*" OR "undernutrition" OR "under nutrition" OR "nutritional status" OR "weight gain" OR "weight loss" OR "underweight" OR "under weight" OR

"growth failure" OR "marasmus" OR "kwashiorkor" OR "stunt*" OR "wasting" OR "weight for age" OR "length for age" OR "weight for length") OR AB ("mortality" OR "death*" OR "died" OR "survival" OR "diarrhea*" OR "diarrhoea*" OR ("respiratory" N3 ("infect*" OR "illness*")) OR "influenza" OR "pneumonia" OR "bronchitis" OR "sepsis" OR "septic" OR "bacteremia" OR "septicemi*" OR "omphalitis" OR (("umbilical cord" OR "umbilicus") N3 infection*) OR "hospitali*" OR ("hospital" N3 "admission*") OR "malnutri*" OR "malnourish*" OR "undernourish*" OR "mal nutri*" OR "under nourish*" OR "undernutrition" OR "under nutrition" OR "nutritional status" OR "weight gain" OR "weight loss" OR "underweight" OR "under weight" OR "growth failure" OR "marasmus" OR "kwashiorkor" OR "stunt*" OR "wasting" OR "weight for age" OR "length for age" OR "weight for length"))
AND
((MH "Infant+" OR "Child, Preschool") OR TI ("infant*" OR "new born*" OR "newborn*" OR "neonat*" OR "baby" OR "babies" OR "child*") OR AB ("infant*" OR "new born*" OR "newborn*" OR "neonat*" OR "baby" OR "babies" OR "child*"))

Database: POPLINE
Date of Search: 2015-12-17
Results: 744 Records
Note: Acknowledgements for assistance with running this search to Debra L. Dickson | POPLINE Manager | Knowledge for Health (K4Health) Johns Hopkins Center for Communication Programs (CCP), 111 Market Place, Suite 310, Baltimore, MD 21202 ddickson@jhuccp.org | www.popline.org | 410-659-6300
Search Strategy:

("breast feed" OR "breast feeds" OR "breast feeding" OR "breast fed" OR breastfe*)
AND
(initiation OR initiate* OR initiating OR timing OR delay*)
AND
(mortality OR death* OR died OR survival OR diarrhea* OR diarrhoea* OR "respiratory infection" OR "respiratory illness" OR "respiratory disease" OR "respiratory infections" OR "respiratory illnesses" OR "respiratory diseases" OR influenza OR pneumonia OR bronchitis OR sepsis OR septic OR bacteremia OR septicemi* OR omphalitis OR "umbilical cord infection" OR hospitali* OR "hospital admission" OR "hospital admissions" OR malnutri* OR malnourish* OR undernourish* OR undernutrition OR "nutritional status" OR "weight gain" OR "weight loss" OR underweight OR "under weight" OR "growth failure" OR marasmus OR kwashiorkor OR stunt* OR wasting OR "weight for age" OR "length for age" OR "weight for length")
AND
(infant* OR newborn* OR neonat* OR baby OR babies OR child*)

Database: LILACS
Date of Search: 2015-12-15
Results: 177 Records
Search Strategy:

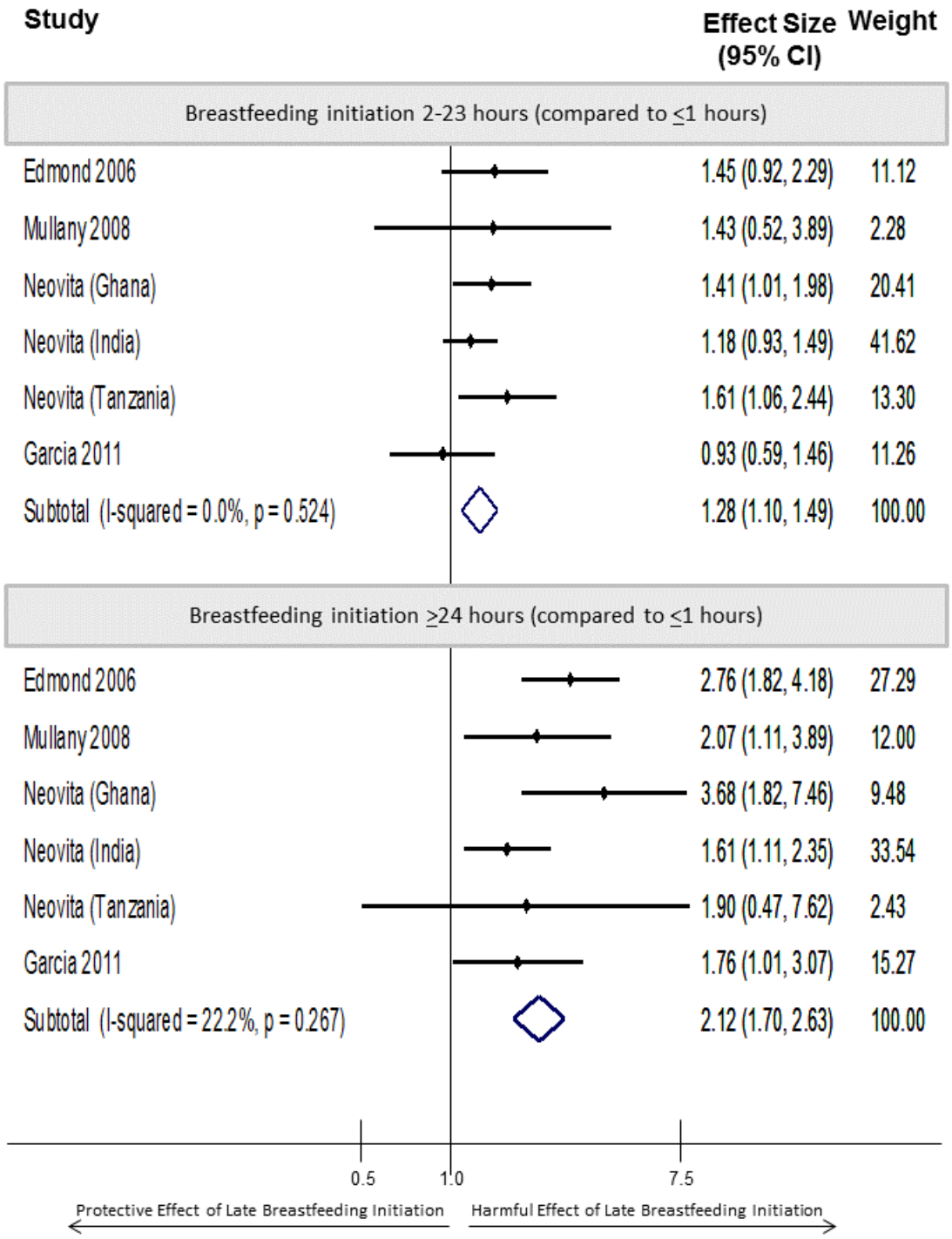
((breast AND feed$) OR breastfe$ OR amamantamiento OR (aleitamento AND materno))
AND
(initiat$ OR timing OR delay$ OR inicia$ OR atrasar OR atraso OR retrasar OR retraso)
AND
(mortality OR death$ OR died OR survival OR diarrhea$ OR diarrhoea$ OR (respiratory AND (infection$ OR illnes$ OR disease&)) OR influenza OR pneumonia OR bronchitis OR sepsis OR septic OR bacteremia OR septicemi$ OR omphalitis OR hospitali$ OR (hospital AND admission$) OR

malnutri$ OR undernurish$ OR undernutrition OR marasmus OR kwashiorkor OR stunt$ OR wasting OR (weight AND (loss OR gain OR age OR length)) OR mortalida$ OR muerte OR supervivencia OR sobrevivencia OR diarrea OR ((infección$ OR enfermedad$) AND irespiratoria$) OR neumonía OR bronquitis OR septicemia OR onfalitis OR (ingres$ AND hospitalar$) OR desnutri$ OR ((aumento OR pérdida OR cambio OR bajo) AND peso) OR debilitante OR morte OR sobrevivência OR diarréia OR ((doença$ OR infecção) AND respiratóri$) OR gripe OR bronquite OR sepsia OR hospitalização OR ((Admiss$ OR internaç$) AND hospitalar$) OR subnutrição OR ((ganho OR perda OR mudança OR baixo OR abaixo OR idade OR comprimento) AND peso) OR nanismo OR desperdiçando)
AND
(infant$ OR newborn$ OR neonat$ OR baby OR babies OR child$ OR niño OR niños OR bebé OR bebés OR ((recién OR recém) AND nascido$) OR criança$)

Database: AIM
Date of Search: 2015-12-15
Results: 6 Records
Search Strategy:
((breast AND feed$) OR breastfe$) AND (initiat$ OR timing OR delay$) [keywords]

Database: Index Medicus for the Eastern Mediterranean Region - IMEMR
Date of Search: 2015-12-15
Results: 37 Records
Search Strategy:
((breast AND feed$) OR breastfe$) AND (initiat$ OR timing OR delay$) [KeyWords]
AND
(mortality OR death$ OR died OR survival OR diarrhea$ OR diarrhoea$ OR (respiratory AND (infection$ OR illnes$ OR disease&)) OR influenza OR pneumonia OR bronchitis OR sepsis OR septic OR bacteremia OR septicemi$ OR omphalitis OR hospitali$ OR (hospital AND admission$) OR malnutri$ OR undernurish$ OR undernutrition OR marasmus OR kwashiorkor OR stunt$ OR wasting OR (weight AND (loss OR gain OR age OR length))) [KeyWords]

Database: WPRI (Western Pacific)
Date of Search: 2015-12-15
Results: 37 Records
Search Strategy:
("breast feeding" OR breastfeeding) AND (initiation OR initiate OR initiates OR timing OR delay OR delays OR delayed)



Appendix 2 - Figure 2A. Forest Plot of the relative risk of neonatal mortality (excluding deaths in the first 2-4 days) for infants who initiated breastfeeding 2-23 hours or ≥24 hours after birth, compared to those who initiated breastfeeding early (<1 or ≤1 hour) – including Garcia 2001 estimates.

Appendix 2 - Table 2A. Summary of Relevant Studies

Study	Population	Study Design	Exposure Definition	Outcomes Definitions	Summary of Limitations	Quality Score
Akter 2015	Bangladesh Demographic and Health Survey	Cross-sectional	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Neonatal Mortality	Inadequate sample size (n=3190). Risk of selection bias due to cross-sectional study design. Risk of information bias due to long recall period (up to 3 years). Did not account for reverse causality.	Very low
Bamji 2008	India: Medak district, Andhra Pradesh	Retrospective (1998-2001) and prospective (2001-03) cohort	Early (<24 hrs) vs. Late (>=24 hrs) breastfeeding initiation	Neonatal Mortality	Risk of selection/attrition bias because 30% of selected women were not reachable for postpartum assessment. Risk of information bias due to long recall (1-2 months after birth). Inadequate adjustment for confounding. Did not account for reverse causality.	Very low
Caglar 2006	Turkey: Gaziosmanpasa Medical Faculty	Case control	Mean age at first breastfeeding (in hours)	Weight loss <10%	No adjustment for confounding.	Very low
Clemens 1999	Egypt : Abu Homos district	Prospective cohort study	Early (<3 days) vs. Late (>3 days) breastfeeding initiation	Diarrhea	Adjusted models do not include a term for preterm or low birthweight status.	Low
Dewey 2003	USA: Davis, California	Prospective cohort	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Excess weight loss at day 3	Inadequate adjustment for confounding. Did not account for reverse causality.	Very low
Edmond 2006	Ghana: Kintampo, Wenchi, Techiman, and Nkoranza districts in the Brong Ahafo region	Prospective cohort (within RCT)	Early (<1 hr) vs. Late (1-24 hr, 24-48 hr, 48-72 hr >=72 hr) breastfeeding iniation	Neonatal Mortality	None	Moderate
Engebretsen 2008	Uganda: Mbale district	Cross-sectional	Early (<2 hr) vs. Late (2-24 hr, >24 hr) breastfeeding iniation	Stunted (HAZ < –2 SD), Wasted (WHZ < –2 SD)	Risk of selection bias. Inadequate adjustment for confounding. Did not account for reverse causality.	Very low

Appendix 2 – Table 2A (continued). Summary of Relevant Studies

Study	Population	Study Design	Exposure Definition	Outcomes Definitions	Summary of Limitations	Quality Score
Enzunga 1990	Zaire: Evangelical Medical Centre	Prospective cohort	Time of breastfeeding initiation (hrs)	Weight loss since birth	The inclusion criteria and loss to follow up are not described. There is no information regarding assessment of exposure or outcome. There is no adjustment for confounding. Did not account for reverse causality.	Very low
Garcia 2011	India: Tamil Nadu	Cohort	Early (<12 hr) vs. Late (12-24 hrs, > 24 hrs) breastfeeding initiation	Neonatal Mortality	None	Moderate
Hajeebhoy 2014	Vietnam: 11 provinces	Cross-sectional	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Diarrhea ARI	Risk of selection bias due to cross-sectional study design. Risk of information bias due to long recall period (up to 3.2 months). Inadequate adjustment for confounding. Did not account for reverse causality.	Very low
Meshram 2012	India: Medak district, Andhra Pradesh	Cross-sectional	Early (<1 hr) vs Late (1-3 hrs, 4-12 hrs, 13-24 hrs, >24 hrs) breastfeeding initiation	Stunted (HAZ < –2 SD), Underweight (WAZ<–2 SD), Wasted (WHZ < –2 SD)	Inadequate sample size. Risk of selection bias due to cross-sectional study design. Inadequate adjustment for confounding.	Very low
Mullany 2008	Nepal: Sarlahi District	Prospective cohort (within a RCT)	Early (<1 hr) vs. Late (1-24 hr, 24-48 hr, 48-72 hr >=72 hr) breastfeeding iniation	Neonatal Mortality	None	Moderate
Mullany 2010	Nepal: Sarlahi District	Prospective cohort (within a RCT)	Breastfeeding initiation (hrs) <24 hrs and >=24 hrs	Hypothermia	None	Moderate
Mullany 2009	Tanzania: Pemba Island	Prospective cohort (within a RCT)	Early (<1 hr) vs. Late* (≥1 hr) breastfeeding initiation	Umbilical cord infection	None	Moderate
Neovita Study Group 2016 [Includes: Edmond 2015 Masanja 2015 Mazunder 2015]	Ghana, India, Tanzania: BrongAhafo Ghana, Haryana India, Dar es Salaam and Morogoro Tanzania	Prospective cohort (within 3 RCTs)	Early (≤1 hr) vs. Late (2-24 hr, >24 hr) breastfeeding iniation	Neonatal Mortality, Mortality from 1-3 months, Mortality from 3-6 months	None	Moderate

Appendix 2 – Table 2A (continued). Summary of Relevant Studies

Study	Population	Study Design	Exposure Definition	Outcomes Definitions	Summary of Limitations	Quality Score
Niswade 2011	India: Nagpur district	Cohort	Delayed initiation of breastfeeding (not defined)	Neonatal Mortality	Did not account for reverse causality.	Very low
Shah 2014	Bangladesh: Sylhet district	Prospective cohort (within RCT)	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Neonatal Mortality	None	Moderate
Sutan 2014	Indonesia: Aceh Province	Unmatched case control	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Neonatal Mortality	Risk of information bias due to restrospective assessment of breastfeeding after child death. Did not account for reverse causality.	Very low
Van den Bosch 1990	Malawi: Kamuzu Central Hsopital	Randomized Control Trial	Early initation encouragement vs. Initation at time of mother's choice	Hypothermia: Rectal temperatue <36.5°C at 2, 4, and ~24 hrs after birth	None. Note that randomized groups were comparable.	High
Wren 2015	Guatemala: Mam-Mayan communities in the Western Highlands	Cross-sectional	Early (<1 hr) vs. Late (>1 hr) breastfeeding initiation	Stunted (HAZ < –2 SD), Underweight (WAZ<–2 SD), Wasted (WHZ < –2 SD)	Inadequate sample size. Inadequate adjustment for confounding.	Very low

Appendix 2 – Table 2B. Quality Assessment of Included Studies

Study	Score for study design	Risk of selection bias?	Risk of information bias?	Risk of attrition bias?	Adequate adjustment for confounding?	Accounted for reverse causality?	Should the score for study design be adjusted for study quality?	Score after adjustment
Akter 2015	Low - Cross-sectional	Yes - cross sectional	Yes - recall up to 3 years allowed	No - not applicable due to study design	Yes	No	Yes	Very low
Bamji 2008	Low - Retrospective and prospective cohort	Yes - 30% of selected women were not reachable and thus excluded from study	Yes - assessment conducted 1-2 months after delivery.	Yes - 30% of selected women were Not reachable and thus excluded from study	No - Proportions presented. No control for confounding.	Yes - excluded 7 children who died on day 1.	Yes	Very low
Caglar 2006	Low - Case control	No	No	No - not applicable due to study design	No	Yes	Yes	Very low
Clemens 1999	Low - Prospective cohort	No	No - median age at enrollment was 11 days (IQR 7-16).	No -loss to follow up = 10%	No - Adjusted models do not include preterm or low birthweight status.	Yes - Median age at enrollment was 11 days.	No	Low
Dewey 2003	Low - Prospective cohort	No	No	No	Not for our exposure and outcome of interest	Not for our exposure and outcome of interest	Yes	Very low
Edmond 2006	Low - Prospective cohort (within RCT)	No	No - median assessment at 14 days postpartum	No	Yes	Yes	Yes	Moderate
Engebretsen 2008	Low - Cross-sectional	Yes - cross sectional	No	No - not applicable due to study design	No	No	Yes	Very low
Enzunga 1990	Low - Prospective cohort	unclear	unclear	unclear	No	No	Yes	Very low

Appendix 2 – Table 2B (continued). Quality Assessment of Included Studies

Study	Score for study design	Risk of selection bias?	Risk of information bias?	Risk of attrition bias?	Adequate adjustment for confounding?	Accounted for reverse causality?	Should the score for study design be adjusted for study quality?	Score after adjustment
Garcia 2011	Low - Prospective cohort	No	No	No	Yes	Yes	Yes	Moderate
Hajeebhoy 2014	Low - Cross-sectional	Yes - cross sectional	Yes - median age as assessment was 3.3 months	No - not applicable due to study design	No	No	Yes	Very low
Meshram 2012	Low - Cross-sectional	Yes - cross sectional	No	No - not applicable due to study design	No	Yes	Yes	Very low
Mullany 2008	Low - Prospective cohort (within RCT)	No	No	No	Yes	Yes	Yes	Moderate
Mullany 2010	Low - Prospective cohort (within RCT)	No	No	No	Yes	Yes	Yes	Moderate
Mullany 2009	Low - Prospective cohort (within RCT)	No	No	No (89% of infants receive all 6 assessments)	Yes	Yes	Yes	Moderate
Neovita 2016 [Includes: Edmond 2015 Masanja 2015 Mazunder 2015]	Low - Prospective cohort (within RCT)	No	No	Very low LTFU	Yes	Early deaths excluded	Yes	Moderate
Niswade 2011	Low - Prospective cohort	No	No - visited on day 0 and day 1	No	Yes	No	Yes	Very low

Appendix 2 – Table 2B (continued). Quality Assessment of Included Studies

Study	Score for study design	Risk of selection bias?	Risk of information bias?	Risk of attrition bias?	Adequate adjustment for confounding?	Accounted for reverse causality?	Should the score for study design be adjusted for study quality?	Score after adjustment
Shah 2014	Low - Prospective cohort (within RCT)	No	No - visisted on day of birth	No (~10% exlcuded due to missing LMP)	Yes	Yes	Yes	Moderate
Sutan 2014	Low - Case control	No	Yes - retrospective assessment of breastfeeding after child death	No - not applicable due to study design	Yes	No	Yes	Very low
Van den Bosch 1990	High - Randomized Control Trial	No	No	No (~11% missing 24 hour measurement)	N/A (due to study design)	Yes - preterm, low birthweight, and low Apgar score infants excluded	Yes	High
Wren 2015	Low - Cross-sectional	Yes - cross sectional	No	No - not applicable due to study design	No	Yes	Yes	Very low

Title: Breastfeeding and Maternal Health among HIV-infected Women in Tanzania

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ABSTRACT

Background: Although breastfeeding has known benefits for HIV-exposed infants, there is conflicting evidence regarding the benefits or harms for HIV-infected, breastfeeding mothers.

Objective: To assess the relationship between feeding practices and the incidence of maternal mortality, morbidity, and indicators of poor nutritional status from six weeks to two years postpartum among women in Tanzania living with HIV.

Methods: Prospective cohort design with Cox proportional hazards models.

Results: At six weeks, three months, and six months postpartum, 85%, 59%, and 13% of women respectively reported exclusively breastfeeding; 91%, 74%, and 25% of women respectively reported any breastfeeding. We found an increased risk of wasting (BMI < 18.5) for women exclusively breastfeeding or continuing any breastfeeding at six weeks, three months, and six months postpartum. Women breastfeeding at six months had a 44% increased risk of wasting (95% CI: 1.00-2.06, p-value 0.05) through two years postpartum. This association was not modified by triple antiretroviral therapy (ART) use. Breastfeeding at six weeks was associated with reduced risk of severe anemia among ART users (adjusted HR 0.28, 95% CI: 0.09-0.86), while it was associated with a non-significant increased risk of severe anemia among non-ART users (adjusted HR 1.95, 95% CI: 0.47-8.06) (p value for interaction 0.04).

Conclusion: Although breastfeeding has proven benefits for infant health, there may be a mixed impact on health outcomes for HIV-infected women. Based on existing evidence, we conclude that there is no clear evidence of strong benefits or harm associated with breastfeeding for HIV-infect women. Additional research is needed to know if HIV-infected women may benefit from nutritional support, in addition to ART, during and after lactation.

Key Words: HIV, Breastfeeding, Mortality, Morbidity, Anemia, Weight Loss

Introduction

UNAIDS estimates that 92% of pregnant women living with HIV reside in sub-Saharan Africa where maternal mortality and HIV prevalence are highest [1]. HIV is known to substantially increase the risk of poor nutritional outcomes and maternal morbidity, and it is estimated to increase maternal mortality by as much as five or ten-fold [2, 3].

Despite recommendations that HIV-infected women receiving antiretroviral therapy exclusively breastfeed infants for six months, and continue breastfeeding through twelve months [4], there is conflicting evidence about the effect of breastfeeding on the risk of maternal mortality and morbidity in the postpartum period. However, some hypothesize that the metabolic demands of lactation, in conjunction with HIV, may result in poor health outcomes for women. A randomized trial of breastfeeding versus formula feeding in Kenya found that mortality among women randomized to breastfeeding was more than three times higher than among women randomized to formula feeding [5]. In contrast, a randomized trial in Botswana found no difference in maternal mortality between breastfeeding and formula feeding groups [6], and a randomized trial conducted in Zambia of prolonged feeding versus abrupt cessation of breastfeeding at four months postpartum also showed no difference in maternal mortality [7]. Additional observational studies [8-10] and a meta-analysis [11] found no significant difference in the risk of mortality for women breastfeeding versus not breastfeeding (comparing ever versus never, or recent feeding versus not).

Similarly, the evidence regarding the relationship between breastfeeding and maternal morbidity is conflicting. The Kenyan trial which noted increased risk of mortality for breastfeeding HIV-

infected women also found increased risk of lactation-associated weight loss within two years of delivery [5]. Another study found significantly faster weight loss and CD4 cell count decline in breastfeeding women [12], while other studies have found no association with nutritional outcomes [9, 13]. Data linking breastfeeding to maternal anemia is limited, although one study suggests that prolonged breastfeeding for six months or more is an independent predictor of postpartum severe anemia in women with HIV [14]. Few studies have examined the relationship between prolonged breastfeeding (six months or more), which is currently recommended for HIV-infected mothers by infant feeding guidelines, and maternal health.

In this study, we examined the relationship between feeding practices and health in a prospective cohort of women living with HIV. Specifically, we assessed the associations between exclusive breastfeeding and any breastfeeding at six weeks, three months, and six months postpartum and mortality, morbidity, and indicators of poor nutritional status in women through two years postpartum.

Methods

Study Design and Population

This prospective cohort consists of women whose children were enrolled in a micronutrient supplementation trial in Tanzania evaluating the effect on infant morbidity and mortality (NCT00197730). The design, implementation, and results of this trial, conducted between August 2004 and November 2007, are described elsewhere [15]. Briefly, women with HIV who presented to antenatal care prior to 32 weeks gestation were recruited to enroll in the trial.

Singleton infants were randomized between five and seven weeks after birth if no congenital abnormality prevented feeding and the mother intended to stay in the study area. Mothers and children were asked to attend monthly clinic visits to receive PMTCT services and participate in the study for two years following enrollment. Initially, antiretroviral therapy was limited to single dose nevirapine prophylaxis for PMTCT. However, in July 2005, women with CD4 cell count <200 cell/uL or WHO stage III and CD4 cell count <350 cell/uL were eligible to begin triple antiretroviral therapy (ART). In accordance with the Tanzanian standard of care at the time of the trial, mothers were counseled on both the risks and benefits of exclusive breastfeeding, and those who chose to exclusively breastfeed were instructed not to give any additional foods or fluids aside from medicines or oral rehydration solutions [15].

Exposure Assessment and Definitions

Breastfeeding was assessed longitudinally by the mother's report during monthly clinic visits. Specifically, the mother was asked if the child consumed any foods from a specified list of 17 items (*e.g.* breast milk, water, cow's milk, formula, juice, etc.) during the previous week. The duration of exclusive breastfeeding and breastfeeding were defined as the midpoint between the last report of breastfeeding or exclusive breastfeeding and the first report of not breastfeeding or not exclusive breastfeeding. Exclusive breastfeeding and any breastfeeding were analyzed as binary exposures at six weeks, three months (90 days), and six months (180 days). Exclusive breastfeeding women were compared to not exclusively breastfeeding women (i.e. predominant, partial, and non-breastfeeding women). Breastfeeding women were compared to non-breastfeeding women.

Outcome Assessment and Definitions

Outcomes of interest included time to death, first hospitalization, first outpatient visit, wasting, anemia, and severe anemia. We included events only if they occurred after the time of exposure assessment. Data regarding morbidity were recorded by study nurses and physicians during study visits. Women were asked if they had been hospitalized or sought healthcare since the last study visit, and the date of hospital admission or outpatient visit was either abstracted from the woman's medical card or reported by the woman.

Weight and height were measured by study nurses at each study visit. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. Excessive weight loss was examined by assessing cases of incident wasting, defined as a BMI of <18.5 [16]. Women with at least two weight measurements occurring 28 days after birth and who did not have a BMI <18.5 at their first postpartum weight measurement were included in the wasting analysis. Women were scheduled to give blood for a complete blood count at baseline, one year, and two years after delivery. Hemoglobin concentration was measured by the AcT5 Diff AL hematology analyzer (Beckman Coulter). Women with at least two measurements and who were not anemic (defined as Hb <11 g/dL) or severely anemic (defined as Hb <8 g/dL) at baseline were included in the anemia and severe anemia analyses. Women without events were censored at the time of their last study visit (maximum of 756 days) or at the time of death of their child.

Statistical Analysis

The cohort was characterized using baseline data regarding household, maternal and infant characteristics by means or proportions for continuous and categorical data respectively.

To examine the relationship between breastfeeding (exclusive breastfeeding or any breastfeeding) and each of the outcomes, Cox proportional hazard models were used [17]. Potential confounders included in the multivariate models were: use of antiretrovirals during pregnancy (yes, no), time-varying CD4 T-cell count (<200 cells/mm^3, 200–349 cells/mm^3, >350 cells/mm^3), birth weight of recent delivery (<2500 grams, ≥2500 grams), gestational age at delivery (<37 weeks, ≥37weeks), whether the woman was *primigravida* (yes , no), woman's education (<primary school completed or ≥primary school completed), and marital status (married or living together vs not). We non-parametrically examined potential non-linear relationships between maternal age and each outcome with restricted cubic splines [18]. We tested non-linearity using the likelihood ratio test comparing the model with the linear term to the model with the linear plus cubic spline terms. In the case that the relationship was neither non-linear nor linear, we included age as a dichotomous variable (<27 years, ≥27 years). We assessed effect modification by antiretroviral use (initiated prior to or during pregnancy and continued thereafter versus not). To determine whether effect modification was statistically significant in the Cox proportional hazard models, we used a likelihood ratio test with one degree of freedom to compare the full model (with interaction term) to the reduced model (without interaction term). The missing indicator method was used for any missing confounders [19]. All hypothesis tests were two-sided, and a p value of <0.05 was considered significant. Analyses were performed using SAS software version 9.2 (Cary, NC).

Ethics

Written informed consent was obtained from all women participating in the parent trial. The trial protocol was approved by the institutional review boards of the Harvard School of Public Health,

Muhimbili University of Health and Allied Sciences, Tanzania Food and Drug Authority, and the Tanzania National Institute of Medical Research.

Results

A total of 2387 women were enrolled in the parent trial, and 2385 women were included in this study. Two women were excluded because one woman never had breastfeeding status ascertained and one woman died prior to the first study visit at six weeks postpartum. The proportion of women exclusively breastfeeding at six weeks postpartum was 85% (n=2035), 59% (n=1399) at three months, and 13% (n=320) at six months. The proportions of women who reported any breastfeeding at six weeks, three months, and six months were 91% (n=2164), 74% (n=1764), and 25% (n=602) respectively. The mean duration of exclusive breastfeeding in the study population was 3.5 months (SD 2.1) and the mean duration of any breastfeeding was 4.4 months (SD 2.5).

The mean age of women at baseline (six weeks postpartum) was 28 years (SD 5). The majority completed at least primary school. More than 60% of all mothers were women without income, and about half were married or living with a partner. Approximately 23% of women were pregnant with their first child. A total of 165 women received triple antiretroviral therapy in pregnancy, and the median CD4 count at baseline was 468 cells/mm^3. Baseline characteristics were similar between those exclusively breastfeeding at six weeks and those who were not (Table 3.1).

A total of 33 women (1.4%) died, 55 women (2.3%) were hospitalized, and 297 women (12.5%) attended a health facility outside of the normal study schedule during the two year follow-up

Table 3.1. Baseline demographic and health characteristics stratified by exclusive breastfeeding status at six weeks postpartum (n=2385)

	Exclusively Breastfeeding at 6 Weeks (n=2035)	Not Exclusively Breastfeeding at 6 Weeks (n=350)
Mean age (y) [mean (SD)]	28 (5)	29 (5)
Gestational age at enrollment (wk) [mean (SD)]	39 (3)	39 (3)
Education [n (%)]		
Did not complete primary school	269 (13.2)	33 (9.4)
Completed primary school or more	1748 (85.9)	313 (89.4)
Employment [n (%)]		
Business woman	104 (5.1)	26 (7.4)
Housewife with income	175 (8.6)	25 (7.1)
Housewife without income	1320 (64.9)	207 (59.1)
Other	372 (18.3)	68 (19.4)
Married/living with partner [n (%)]	1054 (51.8)	175 (50.0)
Prima gravida [n (%)]	454 (22.3)	86 (24.6)
Midupper arm circumference (cm) [mean (SD)]	26 (3)	26 (3)
Hemoglobin		
>11 g/dL [n (%)]	1301 (63.9)	176 (50.3)
Concentration [mean (SD)]	12 (1)	11 (1)
BMI group [n (%)]		
<18.5 kg/m^2	94 (4.6)	17 (4.9)
18.5 to <25 kg/m^2	1219 (59.9)	201 (57.4)
25.0 to <30 kg/m^2	555 (27.3)	96 (27.4)
≥30 kg/m^2	163 (8.0)	163 (8.0)
Received ART during pregnancy [n (%)]	126 (6.2)	39 (11.1)
WHO disease stage I/II [n (%)]	1075 (52.8)	179 (51.1)
CD4 count [median (IQR)]	471 [327-679]	434 [284-640]
CD4 count [n (%)]		
<200 cells/mm	43 (12.3)	155 (7.6)
200–349 cells/mm	63 (18.0)	358 (17.6)
>350 cells/mm	179 (51.1)	1303 (64.0)
Male child [n (%)]	1100 (54.1)	187 (53.4)
Child born < 37 weeks [n (%)]	310 (15.2)	47 (13.3)
Birthweight <2.5 kg [n (%)]	130 (6.4)	31 (8.9)

[a] Percents may not add to 100% due to missing data

period. We found no significant association between exclusive breastfeeding (compared to non-exclusive breastfeeding and no breastfeeding) or any breastfeeding (compared to no breastfeeding) and the risk of death or outpatient visits at six weeks, three months, or six months. However, we found that exclusive breastfeeding at six weeks was significantly associated with a 47% reduced risk of hospitalization (adjusted HR 0.53, 95% CI: 0.29-0.99). Similarly, exclusive breastfeeding at three months was independently associated with a reduced risk of hospitalization (adjusted HR 0.54, 95% CI: 0.29-0.99) (Table 3.2).

We examined ART use during pregnancy as a potential effect modifier for morbidity and mortality outcomes. We found that the reduced risk of hospitalizations associated with exclusive breastfeeding at six weeks was limited to women who did not use ART (adjusted HR 0.39, 95% CI: 0.18-0.82) (p value for interaction 0.03) (Table 3.3). Similarly, the risk of hospitalization associated with exclusive breastfeeding at three months was significantly reduced among women not using ART (adjusted HR 0.45, 95% CI: 0.21-0.94) (p value for interaction 0.04) (Table 3.4).

Most women (n=2318) had at least two postpartum weight measurements taken more than four weeks after delivery. Among these women, 101 (4.4%) had a BMI of <18.5 at baseline and were excluded from the analysis. A total of 2217 women were included in the wasting analysis. Among these women, 277 (12.5%) had a change in BMI to <18.5 over the course of follow-up. Women who were still breastfeeding at six months had a 44% increased risk of wasting compared to women who had stopped breastfeeding (95% CI: 1.00-2.06).

Table 3.2. Exclusive breastfeeding (compared to predominant, partial, and no breastfeeding) or any breasteeding (compared to no breastfeeding) - at six weeks, three months, six months - in relation to maternal mortality, first hospitalization, first outpatient visit, wasting (BMI < 18.5), anemia diagnosis (Hgb < 11g/dL), and severe anemia diagnosis (Hgb < 8g/dL).

			Exclusive Breastfeeding (Compared to Predominant, Partial, or No Breastfeeding)				Any Breastfeeding (Compared to No Breastfeeding)			
			Univariate		Multivariate		Univariate		Multivariate	
Outcome	n events	N total	HR (95% CI)	p value	HR[a] (95% CI)	p value	HR (95% CI)	p value	HR[a] (95% CI)	p value
Breastfeeding at Six Weeks										
Mortality	33	2385	0.50 (0.22,1.10)	0.08	0.60 (0.26,1.37)	0.23	0.50 (0.19,1.28)	0.15	0.62(0.23,1.66)	0.34
Hospitalization	55	2385	0.45 (0.25,0.83)	0.01	0.53 (0.29,0.99)	0.05	0.60 (0.27,1.33)	0.21	0.80 (0.35,1.82)	0.59
Outpatient Visit	297	2385	0.81 (0.60,1.11)	0.19	0.85 (0.62,1.16)	0.30	0.79 (0.54,1.15)	0.22	0.83 (0.57,1.23)	0.35
Wasting[b]	277	2217	1.21 (0.84,1.75)	0.31	1.28 (0.88,1.86)	0.19	1.16 (0.74,1.83)	0.51	1.29 (0.81,2.05)	0.28
Anemia[c]	437	1211	1.07 (0.80,1.43)	0.65	1.05 (0.78,1.41)	0.74	0.98 (0.68,1.40)	0.90	0.98 (0.68,1.41)	0.90
Severe Anemia[d]	71	1709	1.08 (0.54,2.17)	0.84	1.14 (0.56,2.32)	0.71	0.80 (0.36,1.74)	0.57	0.89 (0.40,1.98)	0.77
Breastfeeding at Three Months										
Mortality	29	2196	0.51 (0.24,1.05)	0.07	0.52(0.24,1.12)	0.09	0.49 (0.23,1.04)	0.06	0.51(0.23,1.1)	0.09
Hospitalization	42	2184	0.51 (0.28,0.94)	0.03	0.54 (0.29,0.99)	0.05	0.54 (0.29,1.01)	0.05	0.59 (0.31,1.13)	0.11
Outpatient Visit	227	2128	0.84 (0.64,1.09)	0.19	0.86 (0.66,1.12)	0.27	0.75 (0.56,1.00)	0.05	0.76 (0.56,1.02)	0.06
Wasting[b]	190	2017	1.15 (0.85,1.55)	0.36	1.20 (0.89,1.62)	0.24	1.18 (0.83,1.67)	0.36	1.24 (0.87,1.77)	0.24
Anemia[c]	437	1209	1.15 (0.95,1.40)	0.16	1.15 (0.94,1.40)	0.17	1.08 (0.86,1.36)	0.49	1.08 (0.86,1.37)	0.49
Severe Anemia[d]	71	1704	1.20 (0.73,1.96)	0.47	1.29 (0.78,2.11)	0.32	1.12 (0.63,1.98)	0.70	1.20 (0.67,2.14)	0.53
Breastfeeding at Six Months										
Mortality	25	2031	1.08 (0.37,3.13)	0.89	0.85(0.28,2.6)	0.78	0.67 (0.25,1.79)	0.42	0.5(0.18,1.38)	0.18
Hospitalization	30	2008	0.63 (0.19,2.06)	0.44	0.69 (0.21,2.29)	0.55	0.29 (0.09,0.96)	0.04	0.32 (0.10,1.06)	0.06
Outpatient Visit	176	1918	0.94 (0.61,1.43)	0.76	0.93 (0.60,1.42)	0.72	0.79 (0.56,1.13)	0.20	0.79 (0.55,1.12)	0.19
Wasting[b]	137	1818	1.10 (0.70,1.73)	0.69	1.18 (0.75,1.87)	0.48	1.37 (0.96,1.95)	0.08	1.44 (1.00,2.06)	0.05
Anemia[c]	430	1200	1.06 (0.82,1.37)	0.67	1.08 (0.83,1.41)	0.56	1.02 (0.82,1.26)	0.86	1.04 (0.84,1.29)	0.73
Severe Anemia[d]	68	1699	1.33 (0.73,2.43)	0.36	1.37 (0.75,2.51)	0.31	1.29 (0.77,2.14)	0.33	1.27 (0.76,2.13)	0.36

[a] Time-varying CD4, ARV during pregnancy (yes/no), birthweight (<2500g yes/no), gestational age at delivery (<37 weeks yes/no), prima gravida (yes/no), woman's age (>28 yes/no), woman's education (< primary school completed yes/no)

[b] Wasting defined as BMI < 18.5

[c] Anemia defined as <11 g/dl

[d] Severe anemia defined as <8 g/dl

Table 3.3. Exclusive breastfeeding (compared to predominant, partial, and no breastfeeding) or any breasteeding (compared to no breastfeeding) -**at six weeks** - in relation to maternal mortality, wasting, anemia, severe anemia, first hospitalization, and first outpatient visit during the first 2 years after delivery - stratified by ARV use.

			Exclusive Breastfeeding			Any Breastfeeding		
Outcome	n events	N total	HR[a] (95% CI)	p value	p value interaction	HR[a] (95% CI)	p value	p value interaction
Mortality								
ARV Use	7	428	0.59 (0.09, 3.86)	0.58	0.86	0.36 (0.05, 2.46)	0.30	0.39
No ARV Use	17	1692	0.55 (0.15, 1.96)	0.36		0.94 (0.12, 7.29)	0.96	
Hospitalization								
ARV Use	17	428	2.43 (0.51, 11.60)	0.27	0.03	2.98 (0.38, 23.30)	0.30	0.06
No ARV Use	34	1692	0.39 (0.18, 0.82)	0.01		0.53 (0.20, 1.40)	0.20	
Outpatient Visit								
ARV Use	66	428	0.70 (0.39, 1.27)	0.24	0.26	0.53 (0.28, 1.00)	0.05	0.10
No ARV Use	218	1692	1.12 (0.74, 1.70)	0.59		1.27 (0.72, 2.24)	0.40	
Wasting[b]								
ARV Use	68	402	1.14 (0.60, 2.15)	0.69	0.69	1.18 (0.57, 2.42)	0.66	0.77
No ARV Use	192	1600	1.30 (0.81, 2.09)	0.28		1.32 (0.69, 2.50)	0.40	
Anemia[c]								
ARV Use	72	219	0.71 (0.39, 1.32)	0.29	0.21	0.60 (0.31, 1.17)	0.14	0.10
No ARV Use	359	976	1.19 (0.84, 1.67)	0.32		1.25 (0.79, 1.96)	0.34	
Severe Anemia[d]								
ARV Use	16	350	0.42 (0.14, 1.25)	0.12	0.07	0.28 (0.09, 0.86)	0.03	0.04
No ARV Use	54	1332	1.83 (0.66, 5.08)	0.25		1.95 (0.47, 8.06)	0.36	

a Adjusted for time-varying CD4, birthweight (<2500g yes/no), gestational age at delivery (<37 weeks yes/no), prima gravida (yes/no), woman's age (>28 yes/no), woman's education (< primary school completed yes/no)

[b] Wasting defined as BMI <18.5

[c] Anemia defined as <11 g/dl

[d] Severe anemia defined as <8 g/dl

Table 3.4. Exclusive breastfeeding (compared to predominant, partial, and no breastfeeding) or any breasteeding (compared to no breastfeeding) -**at three months** - in relation to maternal mortality, wasting, anemia, severe anemia, first hospitalization, and first outpatient visit during the first 2 years after delivery - stratified by ARV use.

			Exclusive Breastfeeding			Any Breastfeeding		
Outcome	n events	N total	HR[a] (95% CI)	p value	p value interaction	HR[a] (95% CI)	p value	p value interaction
Mortality								
ARV Use	6	409	[e]		0.16	0.06 (0.01, 0.76)	0.03	0.15
No ARV Use	15	1635	1.10 (0.37, 3.24)	0.87		1.55 (0.34, 6.98)	0.57	
Hospitalization								
ARV Use	12	405	1.25 (0.38, 4.07)	0.72	0.37	1.42 (0.37, 5.45)	0.61	0.45
No ARV Use	28	1629	0.45 (0.21, 0.94)	0.04		0.52 (0.24, 1.14)	0.10	
Outpatient Visit								
ARV Use	49	393	0.74 (0.42, 1.32)	0.31	0.07	0.68 (0.38, 1.24)	0.21	0.25
No ARV Use	170	1588	0.97 (0.71, 1.33)	0.86		0.83 (0.58, 1.17)	0.29	
Wasting[b]								
ARV Use	50	374	1.43 (0.81, 2.53)	0.22	0.81	1.50 (0.80, 2.84)	0.21	0.86
No ARV Use	131	1507	1.05 (0.73, 1.50)	0.81		1.13 (0.73, 1.75)	0.59	
Anemia[c]								
ARV Use	72	218	1.32 (0.82, 2.15)	0.26	0.37	1.07 (0.64, 1.81)	0.79	0.76
No ARV Use	359	975	1.12 (0.90, 1.40)	0.30		1.10 (0.84, 1.43)	0.49	
Severe Anemia[d]								
ARV Use	16	346	0.50 (0.18, 1.41)	0.19	0.04	0.78 (0.27, 2.24)	0.64	0.40
No ARV Use	54	1331	1.66 (0.90, 3.06)	0.10		1.34 (0.65, 2.75)	0.42	

a Adjusted for time-varying CD4, birthweight (<2500g yes/no), gestational age at delivery (<37 weeks yes/no), prima gravida (yes/no), woman's age (>28 yes/no), woman's education (< primary school completed yes/no)

[b] Wasting defined as BMI <18.5

[c] Anemia defined as <11 g/dl

[d] Severe anemia defined as <8 g/dl

[e] Hazard ratio and 95% CI not estimable. No events in the (exclusive) breastfeeding group.

Approximately 73% of women (n=1734) had at least two postpartum complete blood count measurements and were thus eligible for inclusion in the anemia analysis. Among these women, 523 had a hemoglobin count of <11 g/dL at baseline and were excluded from the analysis. Among the 1211 women included in the analysis of anemia, there were 437 (36%) incident cases of anemia over the course of follow-up. Univariate and multivariate analysis showed no relationship between exclusive breastfeeding or any breastfeeding at any time with anemia (Table 3.2).

Of the 1734 women who had at least two postpartum CBC measurements, 25 had a hemoglobin count of <8 g/dL at baseline and were excluded from the analysis of severe anemia. A total of 1709 women were included in the analysis of severe anemia, and 71 women (4.2%) experienced an incident case of severe anemia. Overall, there was no relationship between exclusive breastfeeding or any breastfeeding and severe anemia, but there appeared to be effect modification by ART use (Table 3.2). Exclusive breastfeeding and breastfeeding were associated with a reduced risk of severe anemia among women using ART, while they were associated with an increased risk of severe anemia among women not using ART (Tables 3.3,3.4,3.5). For example, breastfeeding at six weeks (compared to no breastfeeding at six weeks) was associated with a 72% reduced risk of severe anemia among ART users (95% CI: 0.09-0.86), while breastfeeding at six weeks (compared to no breastfeeding at six weeks) was associated with an increased risk of severe anemia among non-ART users (adjusted HR 1.95, 95% CI: 0.47-8.06) (p value for interaction 0.04) (Table 3.3).

Table 3.5. Exclusive breastfeeding (compared to predominant, partial, and no breastfeeding) or any breasteeding (compared to no breastfeeding) -**at six months** - in relation to maternal mortality, wasting, anemia, severe anemia, first hospitalization, and first outpatient visit during the first 2 years after delivery - stratified by ARV use.

			Exclusive Breastfeeding			Any Breastfeeding		
Outcome	n events	N total	HR[a] (95% CI)	p value	p value interaction	HR[a] (95% CI)	p value	p value interaction
Mortality								
ARV Use	4	388	[e]		0.15	[e]		0.12
No ARV Use	15	1568	1.17 (0.32, 4.24)	0.81		0.78 (0.24, 2.51)	0.68	
Hospitalization								
ARV Use	9	381	[e]		0.07	[e]		0.04
No ARV Use	20	1554	1.03 (0.30, 3.52)	0.97		0.51 (0.15, 1.75)	0.29	
Outpatient Visit								
ARV Use	40	363	0.53 (0.16, 1.77)	0.30	0.07	0.55 (0.24, 1.26)	0.15	0.18
No ARV Use	133	1488	1.08 (0.68, 1.71)	0.75		0.86 (0.58, 1.29)	0.47	
Wasting[b]	.	.		.			.	
ARV Use	36	341	1.22 (0.47, 3.21)	0.68	0.86	1.49 (0.73, 3.07)	0.28	0.96
No ARV Use	98	1414	1.13 (0.66, 1.94)	0.65		1.37 (0.89, 2.10)	0.15	
Anemia[c]								
ARV Use	71	216	1.58 (0.80, 3.12)	0.19	0.25	0.96 (0.55, 1.68)	0.88	0.71
No ARV Use	353	968	1.01 (0.76, 1.35)	0.95		1.06 (0.84, 1.34)	0.62	
Severe Anemia[d]								
ARV Use	15	344	0.80 (0.17, 3.74)	0.77	0.43	0.66 (0.18, 2.41)	0.52	0.18
No ARV Use	52	1328	1.52 (0.78, 2.96)	0.22		1.56 (0.88, 2.76)	0.13	

a Adjusted for time-varying CD4, birthweight (<2500g yes/no), gestational age at delivery (<37 weeks yes/no), prima gravida (yes/no), woman's age (>28 yes/no), woman's education (< primary school completed yes/no)

[b] Wasting defined as BMI <18.5

[c] Anemia defined as <11 g/dl

[d] Severe anemia defined as <8 g/dl

[e] Hazard ratio and 95% CI not estimable. No events in the (exclusive) breastfeeding group.

Discussion

Our study identified mixed health effects associated with breastfeeding among HIV-infected women in Tanzania. Breastfeeding was weakly associated with increased risk of wasting among all women and increased risk of severe anemia among women without ART access. We also found lower risk for hospitalization among women who exclusively breastfed for three months or more. We found no increased risk of mortality in relation to breastfeeding among this study population; this is consistent with other observational data [9-11].

Undernutrition and anemia are major public health problems in low-income countries and contribute substantially to maternal morbidity and mortality; they are associated with disease progression and decreased survival in people living with HIV [20-27]. We found a significant, elevated risk of wasting (BMI <18.5) associated with breastfeeding for six months or more, and similar, although non-significant, risk estimates associated with exclusive breastfeeding and breastfeeding at other time points. This relationship was not modified by ART use. We saw the highest risk of wasting among women breastfeeding six months or more, which is consistent with research showing that lactation-associated fat mobilization and weight loss increase substantially after the first three months postpartum [28]. Similarly, HIV-infected, breastfeeding women in Kenya experienced faster weight loss compared to non-breastfeeding peers [12]. Although a recent meta-analysis including studies from high- and low-income countries found no clear relationship between breastfeeding and maternal weight change [29], there is some evidence to suggest that women with HIV may be more susceptible to lactation-associated weight loss. For example, among a cohort of breastfeeding women in South Africa, women with HIV had a mean weight loss of 1.4 kg, compared to 0.4 kg weight gain in HIV-uninfected

women between eight and 24 weeks postpartum [30]. In contrast, studies from Tanzania and Zambia found no relationship between breastfeeding and wasting among cohorts of HIV-infected women [9, 31]. Additional research is needed to understand the relationship between breastfeeding—especially prolonged breastfeeding (six months or more)—and the risk of excess weight loss among HIV-infected women. Pre-pregnancy health and nutritional status, as well as postpartum dietary intake, should be carefully incorporated into future analyses.

Concerns about poor maternal health among breastfeeding women with HIV are supported by the concept of reproductive stress or "maternal depletion syndrome," which suggest that pregnancy and lactation may cause poor health outcomes for women [32]. Though limited research suggests that metabolic adaptation allows lactating women to maintain energy balance, even in the presence of illness or undernutrition [33, 34], it is not clear if this is true for HIV-infected women. In fact, resting energy expenditure has been shown to be higher in HIV-infected women when compared to HIV-uninfected women [35]. Further, there is no specific data regarding resting energy expenditure or total daily energy expenditure among pregnant and lactating women with HIV who are subject to additional metabolic demands [36].

Pregnant women are at high risk for anemia [37], and women with HIV are particularly susceptible due to HIV infection itself and AIDS-related infections [22, 23]. However, the relationship between breastfeeding and anemia risk in women with HIV is not well-studied. We found breastfeeding was associated with an increased risk of severe anemia among women who did not use ART and a reduced risk of severe anemia among women who received ART. In general, ART reduces the risk of anemia [21, 38, 39], although regimens containing Zidovudine

(AZT) and Efavirenz (EFV) may increase the risk of anemia [40]. Our results are consistent with a multi-site trial conducted in Burkina Faso, Kenya, and South Africa, the Kesho Bora Trial, which found that prolonged breastfeeding (six months or longer) was associated with a 62% increase in the risk of severe anemia through 18 months postpartum compared with women who breastfed for less than six months; although some of these women were randomized to ART during pregnancy, they ceased use of ART at the time of breastfeeding cessation (up to six months postpartum) [14]. In addition to ART use, the relationship between breastfeeding and anemia is likely modified by baseline hemoglobin status and other components of antenatal care such as provision of iron-fortified replacement feeding, maternal use of anti-helminthics, intermittent preventive treatment of malaria, and micronutrient supplementation. Additional research regarding the interaction between breastfeeding, ART use, and anemia in postpartum women is needed.

Breastfeeding was associated with a reduced risk of hospitalization in our study. However, our results must be interpreted cautiously since hospitalization is a measure of both morbidity and care-seeking behavior. There is evidence of longer-term health benefits for women who have breastfed, including reduced risk of type 2 diabetes [41], hypertension [42], hyperlipidemia [42], heart disease [42], breast cancer [29, 43], and ovarian cancer [29, 44]. But there is no clear biological mechanism to explain reduced risk of hospitalization among breastfeeding women in our study. We hypothesize that maternal reports of hospitalization may be linked to seeking care for the infant. Breastfeeding has significant infant health benefits [45-48], so the observed reduced risk of hospitalization may be the result of improved infant health. Further, measurement

of hospitalization, which is self-reported, is more susceptible to measurement error; it is not an objective health measure, such as body weight or hemoglobin status.

Due to the observational study design, we cannot rule out residual confounding or the possibility that sicker women chose to stop breastfeeding (*i.e.* reverse causality). In addition, we did not assess breastfeeding and maternal health outcomes during the first six weeks postpartum. Additionally, we conducted multiple statistical tests which increases the probability that some of our findings may have occurred by chance. There are limitations in using observational data, but additional randomized trials to assess maternal health outcomes are unlikely due to current guidelines that promote breastfeeding given its clear benefits for HIV-exposed infants [4]. However, our study's longitudinal design—with repeated assessment of all outcomes, and follow up until two years postpartum—is one of its strengths. Furthermore, breastfeeding was carefully assessed at monthly study visits, which limits recall bias. Finally, the sample size and number of events is large compared to previous research.

Although breastfeeding has proven benefits for infant health, there may be a mixed impact on health outcomes for HIV-infected women. Based on existing evidence, we conclude that there is no clear evidence of strong benefits or harm associated with breastfeeding for HIV-infect women. Additional research is needed to know if HIV-infected women may require nutritional support, in addition to ART, during and after lactation.

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Authors' contributions

This project was a collaboration including Emily R. Smith (ES), Karim Manji (KM), Ronald J. Bosch (RB), Donna Spiegelman (DS), Chris Duggan (CD), Nan Li (NL), Said Aboud (SA), Roger Shapiro (RS), and Wafaie Fawzi (WF). KM and CD were involved in the field activity and supervision of the clinical trial from the onset until completion. ES, WF were involved in the conception of this secondary analysis. ES, NL, RB, DS developed the analysis plan. ES analyzed the data. ES, WF, RS interpreted the data. ES drafted the manuscript. All collaborators reviewed, revised, and approved the final manuscript.

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